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THE EFFECT OF THE SOCIAL ENVIRONMENT ON THE PARENTAL CARE AND OFFSPRING PERFORMANCE OF BURYING BEETLES, *NICROPHORUS VESPILLOIDES*.

Submitted by Olivia Box Power, to the University of Exeter as a thesis for the degree of Masters by Research in Biological Sciences, February 2017.

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ABSTRACT

This thesis details two experiments on burying beetles, *Nicrophorus vespilloides*, examining the evolutionary consequences of different elements of the social environment for parental care and offspring performance.

The first experiment (Chapter 2) followed on from a previous study which found that, under biparental conditions, females burying beetles from lines selected for a high mating rate provide less parental care and have lower offspring performance than those from a low mating rate line. This suggested that selection on females in the high mating rate line due to the costs of mating lead to the evolution of a reduction in their parental care. In contrast, selection on mating rate had no effect on male parental care, suggesting the costs to a high mating rate are greater for females than males. Male and female burying beetles in the wild also commonly display uniparental care, where they care for their larvae in the absence of a partner. Chapter 2 investigated whether the costs to a high mating rate for parental care are also greater for females than males under uniparental conditions. The results of the experiment suggest that the costs of a high mating rate are indeed higher for females than males under uniparental conditions. Few studies have investigated the impact of the social environment on parental care across different non-social environments. The other aim of this experiment was to investigate whether the impact of a high mating rate (social environment) on parental behaviour was dependent upon the non-social environment (carcass size). This is important because, if this is the case, the evolution of plasticity in parental care in response to the social environment will be heavily impacted by the non-social environment experienced (e.g. resource availability). The size of the carcass on which burying beetles breed is an important element of their non-social environment, as it has a large impact on the number of larvae that can be raised and on larval mass. Costs of a high mating rate on the number of larvae initially produced and the proportion of larvae surviving to dispersal were found to be greater when breeding on a large carcass, suggesting high mating rate line individuals are unable to take advantage of the extra resources available.

Few studies have investigated the impact of female-female competition for resources on the expression of parental care, especially in species other than mammals. One of the reasons that female-female competition has received little attention is that it was perceived to be of less evolutionary significance than male-competition, as in many species males show more aggression and have more elaborate ornaments and weapons. However, in recent years the significance of female-female competition for female fitness has become more appreciated. The second experiment in this thesis (Chapter 3) investigated the impact of the presence of a rival female prior to reproduction on female parental care and offspring performance. An earlier study had found that female burying beetles that experience competition increase their expression of parental care. I therefore predicted in my study that females may be able to alter the phenotype of their offspring to best match them to the competition environment they are likely to experience. My study differed from the previous study by using beetles from genetically diverged selection lines. This enabled me to investigate whether there is a genetic variability for this plasticity in parental care behaviour (a GxE) in response to the social environment. Whether there is a GXE for plasticity in behaviour has important implications for the speed at which the extent of plasticity will evolve in response to selection. The results of my experiment showed that females that experienced a rival produced heavier offspring. This is potentially an example of an anticipatory parental effect, as heavier larvae are expected as adults to be better able to compete for access to a carcass. There was no GxE for plasticity in parental care behaviours, suggesting that the extent of plasticity of parental care behaviours may not evolve quickly in response to selection, such as due to climate change.

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AUTHOR'S DECLARATION

This study details two experiments conducted on burying beetles. Prior to my arrival at the university, Mauricio J Carter and Nick Royle designed the experiment detailed in Chapter 2, and Mauricio J Carter collected the data. I then sorted the data and decided how to analyse it. I designed, collected and analysed the data from the experiment in Chapter 3.

CHAPTER 1: GENERAL INTRODUCTION

PARENTAL CARE

Parental care incorporates a wide range of parental behaviours, ranging from provisioning of eggs to the feeding and guarding of juveniles (Smiseth et al. 2012). These behaviours can have both short and long term benefits for parent and offspring fitness, by increasing offspring survival and chances of reproduction (Alonso-Alvarez & Velando 2012). Short term benefits include a parent guarding its offspring from a predator, preventing it from dying as a juvenile (e.g. mouth-brooding St Peter's fish (Balshine-Earn & Earn 1998; Alonso-Alvarez & Velando 2012)). Long term benefits of parental care include a juvenile being fed by a parent, consequently increasing its size as an adult. This increase in adult size could make it more successful in competition for resources and more likely to produce offspring (Alonso-Alvarez & Velando 2012). The benefits of parental care can be quantified. For example, in the burying beetle *Nicrophorus vespilloides*, parental care has been shown to increase offspring mass (Eggert et al. 1998), making them more likely as an adult to win contests against other individuals for access to a carcass on which to breed (Otronen 1988). In some species parental care is essential for offspring survival (e.g. mammals (Balshine 2012)), whereas other species provide no parental care at all (e.g. 80% of bony fish families (Gittleman 1981)). Benefits of parental care can vary depending on the environment experienced by offspring. For example, male parental care increases offspring survival in the California mouse, *Peromyscus californicus*, in cold temperatures but not in warm temperatures (Gubernick et al. 1993). It is also possible for the benefits of parental care to differ between male and female offspring. Adult male red deer, *Cervus elaphus*, reproductive success is more variable than that of females, and is more affected by their growth and the parental care they received during infancy (Clutton-Brock et al. 1981). The causes and consequences of variation both within and between species in the expression of parental care is still poorly understood. Although parental care can be beneficial to offspring development and survival, it can come at a cost to a parent's own survival and residual reproductive value (potential to produce future offspring) (Alonso-Alvarez & Velando 2012). For example, a study on the burying beetle *Nicrophorus orbicollis* increased female investment in parental care by manipulating them to

produce excess offspring. This was achieved by allowing females to breed and lay eggs on a large carcass before it was replaced with a smaller carcass. The study found that females that invested more in parental care showed a greater loss in mass over each breeding round, had a shorter lifespan, and produced fewer offspring over their life time (Creighton et al. 2009). Energetic, mechanistic and time costs of parental care can result in trade-offs between investing in different elements of parental care (such as pre- and post- hatching care), and between investing in current and future offspring (Trivers 1972). Of particular interest is how the expression and evolution of parental care is dependent upon the social environment experienced, such as the parental care behaviour of a mate or competition with conspecifics, and how parental care coevolves with traits expressed by other individuals. This is because parental care can involve many social interactions with conspecific individuals, especially family members, making them a significant element of the social environment experienced by an individual and important for the expression and evolution of parental care.

THE IMPORTANCE OF THE SOCIAL ENVIRONMENT FOR PARENTAL CARE EXPRESSION AND EVOLUTION

The expression of parental care as well as many other behaviours is often highly dependent upon the environment experienced, which can be divided into the non-social and social environment. Elements of the non-social environment are conditions such as temperature, water and food availability, presence of predators or prey and the availability of shelter. The social environment concerns the presence and behaviour of other members of the same species. This could include the demand for care from offspring, the presence of rival individuals in competition over access to resources, and the mating behaviour and parental care of a partner. A key aspect of the social environment is that it can evolve (Royle et al. 2014; Dingemanse & Araya-Ajoy 2015), which can be described through the framework of indirect genetic effect (IGEs). IGEs occur when the genes expressed in one individual affect the phenotype expressed by another individual. This contrasts with direct genetic effects (DGEs), which are the effect of an individual's own genes on their own phenotype (Wolf 2000; McGlothlin & Brodie III 2009; Dingemanse & Araya-Ajoy 2015). For example, the effect of IGEs on the expression of aggression behaviour have been found

in numerous species, ranging from deer mice, *Peromyscus maniculatus,* (Wilson et al. 2009) to fruit flies, *Drosophila melanogaster* (Saltz 2013). IGEs can evolve in response to selection, meaning that the social environment experienced can evolve (Moore et al. 1997; McGlothlin et al. 2010; Dingemanse & Araya-Ajoy 2015). The correlation between the direct and indirect genetic effects on a trait can affect the rate of evolutionary change; a positive correlation can accelerate the response to selection, while a negative correlation can inhibit it (Moore et al. 1997; Wolf 2003; Dingemanse & Araya-Ajoy 2015). IGEs can lead to the coevolution and coadaptation of traits expressed in parents and offspring. For example, a cross-fostering experiment on burrower bugs, *Sehirus cinctus*, revealed a negative genetic correlation between female provisioning of offspring and offspring begging; females that were less generous at provisioning had offspring that were better able to elicit food from a foster mother. A female burrower bug's provisioning is therefore influenced by gene expression in both herself (DGEs) and her offspring (IGEs) (Agrawal et al. 2001).

One important element of the social environment in relation to the expression of parental care in many species is the mating traits of a partner. Investment in mating traits can be costly for an individual, and this can lead to a trade-off between mating traits and parental care (Stiver & Alonzo 2009). Investment in mating traits can include investment in ornaments in order to attract a mate (e.g. both sexes of crested auklet seabird, *Aethia cristatella*, have a forehead crest (Jones & Hunter 1993)), or repeated mating rate to reduce sperm competition from rival males (e.g. burying beetles, *Nicrophorus vespilloides* (Müller & Eggert 1989; House et al. 2008)). Costs to mating traits include increased risk of predation, infection and energetic costs (Daly 1978; Rowe 1994; Chapman et al. 1995; Davies et al. 2012). A study on collard flycatchers, *Ficedula albicollis*, found a trade-off in males between investment in a secondary sexual character (size of a white patch on forehead) and parental effort; males that raised an experimentally increased brood had a smaller forehead patch the following year, which may reduce their future reproductive success (Gustafsson et al. 1995). Differences between males and females in the costs and benefits of mating traits can lead to sexual conflict, which occurs when the optimum trait values of the sexes do not equal (Chapman et al. 2003; Chapman 2006). For

example, optimum mating rate is often lower for females than for males. In fruit flies, *Drosophila melanogaster*, seminal fluid proteins, produced in the male accessory glands, are beneficial for males as they decrease female receptivity to future mating and remove sperm of previous males. However, these products are costly to females, causing a reduction in their lifespan, indicating potential sexual conflict between males and females over mating rate (Chapman et al. 1995). Selection on the mating traits and parental care of one sex can therefore have a significant effect on the expression and evolution of these traits in the other (Alonzo 2010). Understanding the connections between mating traits and parental care requires measuring the benefits, costs and fitness consequences of these traits in both sexes. Costs of a high mating rate could reduce an individual's fitness by a) reducing the number of offspring they produce (either in the current breeding round or in a future breeding round), b) reducing their own lifespan, (hence reducing the number of future broods they can produce) or c) resulting in them producing low quality offspring who are less likely to reproduce, for example smaller offspring who have a low chance of attracting a mate (Smiseth et al. 2012). Few studies have investigated these costs and benefits of mating traits and parental care across different non-social environmental conditions, which is important for fully understanding how these traits will evolve. For example, in *Drosophila melanogaster*, mating frequency only has a negative effect on female fitness at higher nutrition levels (Chapman & Partridge 1996).

Another important element of the social environment in relation to parental care is the presence of same sex competitors. Both males and females can compete with members of their own sex for mates or access territory or resources required to reproduce (Clutton-Brock 2007; Stockley & Campbell 2013). Much research has been carried out regarding sexual selection on males, such as the evolution of weapons for fighting other males for access to mates (Kruuk et al. 2002; Emlen 2008; Emlen et al. 2012). Less research has been carried out regarding competition between females, despite its likely important consequences for fitness (Stockley & Campbell 2013). For example, female chimpanzees compete with each other for access for food, which has a large effect on their reproductive success (Pusey et al. 2013). To date, most research on female-female competition has focused on mammals, particularly

cooperatively breeding species. Research is therefore required in female-female competition across a broader range of taxa, including insects (Stockley & Campbell 2013). In many species, females invest more in the rearing of offspring than males. This makes it particularly important to research female-female competition to investigate whether investment in competition with other females is constrained by a trade-off with investment in parental care (Clutton-Brock 2007; Stockley & Campbell 2013).

MECHANISMS FOR RESPONDING TO A VARIABLE ENVIRONMENT

Organisms need to be able to be responsive in their phenotype, including in their expression of parental care behaviour, to changes in their environment. This can be over an evolutionary time scale, such as gradual changes in climate leading to the evolution of changes in morphology. However, many aspects of the environment can change over shorter time periods, and require a response over an individual organism's lifetime (Simons 2011). One mechanism for responding to a variable environment is through phenotypic plasticity. This occurs when the same genotype produces different phenotypes under different environmental conditions. Many different traits, ranging from morphology to behaviour, can be plastic in relation to the social or non-social environment (West-Eberhard 1989). Individuals can differ in the extent of their plasticity of a particular trait, and if this difference has a genetic basis there is said to be a gene by environment interaction (a GxE) (Nussey et al. 2007; Dingemanse & Wolf 2013). A GxE means that the extent of plasticity of the trait is heritable, and therefore able to respond to selection (Nussey et al. 2007; Dingemanse & Wolf 2013). A study on burying beetle (*Nicrophorus vespilloides*) lines selected for high or low mating rate found evidence of a GxE for plasticity in male activity on a carcass in relation to the social environment (the presence of a rival male). Males from the low mating rate lines showed a decrease in activity in the presence of a rival, whereas those from the high mating rate lines showed an increase (Carter et al. 2015).

Parental care can be highly plastic in relation to variation in the social environment (Royle et al. 2014). For example, House sparrows (*Passer domesticus*) are plastic in their provisioning of offspring in response to numerous elements of the social environment, including their partner's

behaviour and offspring number and age (Westneat et al. 2011). Males and females can differ in their plasticity in parental care, making it important to investigate plasticity in parental care in both sexes. For example, male house sparrows are more plastic in response to differences in brood size than females (Westneat et al. 2011). While a number of studies have shown that parental care can be plastic to various elements of the social and non-social environment (e.g. Westneat et al. 2011), fewer have investigated whether there is genetic variability (a GxE) for this plasticity (Royle et al. 2014). This has important implications for whether plasticity in parental care can evolve in response to selection, such as due to a change in the environment due to climate change, or in response to selection on the behaviour of the other sex. Studies to investigate whether there is a GxE in plasticity in parental care either need to measure the behaviour of individuals (repeated measures), or populations of genetically diverged lines, in two or more environments (Nussey et al. 2007).

One example of plasticity in parental care is that of parental effects (Mousseau & Fox 1998; Marshall & Uller 2007; Uller 2012). These occur when offspring phenotype is influenced by the phenotype of the parent, such as through their parental care or provisioning of yolk, mRNA or hormones into eggs, rather than through inheriting genes from them (Mousseau & Fox 1998; Uller 2012). Some parental effects, known as 'anticipatory parental effects', are adaptive and increase both parent and offspring fitness (Marshall & Uller 2007; Uller 2012; Uller et al. 2013).This is achieved by parents passing on information to their offspring so as to adapt them to the environment they are likely to experience. Anticipatory parental effects are expected to evolve when the parent has good access to information as to the environment that their offspring will experience, and the costs to passing this on to offspring (plasticity) are low (Marshall & Uller 2007; Hoyle et al. 2012; Uller 2012). Offspring will therefore have highest fitness when their environment matches that experienced by their parent (Uller et al. 2013). For example, late juvenile survival is highest in the insect *Forficula auricularia*, when the availability of food an offspring experiences matches that experienced by their mother (Raveh et al. 2016). A meta-analysis of studies that measured offspring fitness in both environments matching and not matching that experienced by the parent, found only weak evidence for anticipatory

parental effects (Uller et al. 2013). The authors of this analysis and others suggest that this is due to studies not ensuring that the parental environment they are manipulating is a reliable predictor of the future environment experienced by offspring (Uller et al. 2013; Burgess & Marshall 2014). Anticipatory parental effects involve both the parent and offspring displaying plasticity, both of which could potentially respond to selection; the parent phenotype is plastic in response to the external environmental cue (such as predator density), and the offspring phenotype is plastic in response to variation in the parental phenotype (Mousseau & Fox 1998; Uller 2012; Uller et al. 2013). For this plasticity to evolve there needs to be genetic variation for it (a GxE). To date it is unknown whether anticipatory parental effects evolve predominantly through changes in the parental or offspring plasticity (Uller 2012). Knowledge of whether plasticity in parental care involved in parental effects is heritable is therefore informative for answering this question.

Another mechanism for responding to a variable environment other than plasticity is through bet-hedging. This occurs when a particular phenotype has a high long term fitness due to it having low variability in fitness across generations, even though it doesn't have high fitness over a single generation (Simons 2011). There are two types of bet-hedging traits; conservative and diversifying. Conservative bet-hedging traits are those that produce a single phenotype which has a low probability of failure. In contrast, diversifying bet-hedging traits are those in which multiple phenotypes are produced, such as producing offspring of a range of different sizes (Simons 2011). However, it is unknown whether the extent of diversification bet-hedging can vary depending upon the environment experienced. For example, some environments, such as a low population density, may be consistent over longer periods of time, and therefore the need for bet-hedging may be low. In contrast, other environments, such as a very high population density, may be less consistent, meaning that there is less certainty about what the future environment will be, and hence more need for bet-hedging. In addition, the links between plasticity and bet-hedging are as yet unclear. For example, is there a trade-off between the extent of plasticity and bet-hedging for a trait? A recent study on germination in the plant *Lobelia inflata* suggests that this may be the case (Simons 2014).

BURYING BEETLES AS A MODEL SYSTEM FOR INVESTIGATING THE EVOLUTIONARY CONSEQUENCES OF THE SOCIAL ENVIRONMENT FOR PARENTAL CARE

Burying beetles, *Nicrophorus vespilloides*, are an excellent species in which to study the effect of the social environment and its evolutionary consequences for parental care. These beetles breed on a small vertebrate carcass, which they strip of fur and process with secretions before burying it in the soil (Eggert & Müller 1997; Scott 1998; Royle et al. 2013). Larvae hatch in the surrounding soil and crawl on to a crater on the carcass, prepared by the parents, to feed. Both male and female burying beetles can provide care for larvae either alone (uniparental care) or together (biparental care). Burying beetles are unique amongst insects in the extent and variety of the parental care they provide, which can consist of direct care such as feeding larvae, and indirect care such as carcass maintenance and guarding of offspring (Eggert & Müller 1997; Scott 1998; Royle et al. 2013). There are sex differences in their parental care, with males having a tendency to provide less direct care than females (Walling et al. 2008). A burying beetle's fitness is impacted by the number of offspring they initially produce, larval survival, larval mass (quality), and their own condition and ability to successfully raise future broods at the end of the breeding round (Eggert & Müller 1997; Royle et al. 2013). Burying beetles with high fitness produce a large number of high quality offspring, and are in good condition to start future breeding rounds.

The social environment has important consequences for each stage of the burying beetle life cycle. Parental care has an important positive effect on larval growth and survival, whilst sibling competition can have the opposite effect (Eggert et al. 1998). Larval growth is a large determinant of adult beetle size. Adult burying beetles compete with other members of their sex over access to a carcass on which to breed, with the larger individual nearly always winning, making the presences of rival individuals an important element of the social environment (Otronen 1988). Finally, the parental care provided by a partner can have important consequences for an individual's own parental behaviour. Male burying beetles are plastic in their parental care, and provide more direct care in the absence of a partner (Smiseth et al. 2005). All together, this means there are many elements of the burying beetle social environment that can be

experimentally manipulated in order to investigate their consequences for the evolution of their behaviour and life-history. In addition, one of the most important elements of a burying beetle's non-social environment is the size of the carcass on which they breed, which can be easily manipulated, allowing one to investigate the interactions between various elements of the social and non-social environment.

While a number of studies on burying beetles have investigated the effect of male-male competition, less is known about the consequences of female-female competition for parental care. However, a recent study by Pilakouta et al. (2016) found that the presence of a rival female prior to breeding resulted in females spending more time providing direct care and producing larger broods. The authors suggest that this is due to females that experience competition investing more in the current brood, as the probability of breeding in the future may be low. Investigating plasticity in female parental care in response to the competition environment in burying beetles and its impact on fitness is therefore likely to be an informative area of study. In particular, is there genetic variation (a GxE) for this plasticity in parental care in response to the presence of a rival? Is the competition environment that a female experiences a reliable indicator of the competition environment her offspring will experience? Can females, through a maternal effect, adaptively match the mass of their offspring to the competition environment they are likely to experience? Studies on burying beetles have also investigated the effect of mating traits on the expression of parental care. Using burying beetle lines selected for either high or low mating rate, increased mating rate was found to have no effect on male parental care (even though it is expected to increase their paternity), but was found to decrease female parental care, suggesting that the costs to mating are greater for females than males (Head et al. 2014). However, it is unknown how the costs of mating affect parental care in males and females under uniparental conditions. This has important implications for understanding how selection on the mating rate or parental care of one sex will impact fitness and selection on these traits in the other.

AIMS OF THIS PRESENT STUDY

The aim of this study was to investigate the impact of the social environment on burying beetle parental care and larval performance. The focus of the first part of the study was to investigate whether there are sex differences in the cost of a high mating rate on parental care and larval performance under uniparental care conditions, and whether these costs depend on the non-social environment experienced (size of carcasses). I investigated whether the costs of a high mating rate reduce the frequency at which burying beetles can provide parental care, and whether this leads to a reduction in offspring survival and mass, which are two important elements of burying beetle fitness. This study differs from Head et al. (2014) by focusing on the effect of mating rate on parental care in uniparental conditions rather than biparental conditions. This is important, as burying beetles often raise larvae without the aid of a partner, so understanding how mating rate effects the parental care of offspring performance under uniparental conditions is necessary for predicting how the evolution of mating traits will impact parental care in this species. Following from Head et al. (2014), I predicted that the costs of a high mating rate would be greater for females than for males. An additional element of this experiment was to investigate how the costs of mating depend on the non-social environment experienced (i.e., the size of the carcass). On small carcasses demands for parental care from larvae could be relatively high, and the parent would be less able to recoup costs by consuming the carcass themselves. However, the costs of a high mating rate may prevent individuals taking full advantage of breeding on a large carcass. Understanding how the non-social environment affects the impact of the social environment is again important for understanding how mating traits and parental care behaviour of males and females will evolve. Finally, this study also investigated whether there are trade-offs between different elements of parental care.

The focus of the second part of this project was on a different element of the burying beetles' social environment. I investigated whether there is phenotypic plasticity in female parental care in relation to the competition environment she experiences (the presence or absence of a rival female). Few studies to date have investigated the impact of the competition environment on female parental care and offspring performance in burying beetles. I predicted that females

would be able to adaptively match offspring phenotype to the competition environment they are likely to experience. Under high competition conditions, a large size is beneficial for winning carcasses from rival individuals. In contrast, a smaller size may be beneficial for locating carcasses when there is low competition and carcass availability (Hopwood et al. 2016). I therefore predicted that females that experienced competition would produce heavier larvae, as they would be better able as adults to compete for carcasses. This will further our understanding of maternal effects in response to the competition environment, and the extent that females can produce offspring adapted to the environment they are likely to experience. This study also aimed to investigate whether there are trade-offs between different aspects of parental care, such as between investing in offspring size vs offspring number. By using females from genetically diverged lines, I was able to investigate whether there is a gene by environment interaction for this plasticity and therefore whether it would respond strongly to selection. This will be informative more generally for understanding whether plasticity in parental care traits is likely to evolve in response to selection. This has important implications for understanding, for example, whether plasticity in parental care will evolve quickly in response to a change in the environment. An additional novel element of this experiment was to investigate how the extent of a potential diversifying bet-hedging trait, within-brood variation in larval mass, depends on the social environment experienced. If a female doesn't experience a rival female prior to breeding it could signal that her offspring will very likely experience a low population density, and that there is therefore little need for bet-hedging. In contrast, if a female does experience a rival female, there could be more variation in the social environment that her offspring will experience (i.e., the population density they experience could be anywhere from moderate to high). Therefore, the extent of within-brood variation in larval mass could be increased for females that experience a rival. Both of the experiments in this study will further our understanding of the impact of the social environment on the expression and evolution of parental care.

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CHAPTER 2: CONFLICT AND TRADE-OFFS BETWEEN MATING RATE AND PARENTAL CARE

ABSTRACT

Mating traits and parental care are closely connected. For example, an increase in mating rate by males can be costly to females, resulting in a reduction in their parental care behaviour. Costs of mating traits and parental behaviour can sometimes differ between sexes and depend on resource availability. A recent study on burying beetles, *Nicrophorus vespilloides*, by Head et al. (2014) found that artificial selection for a high mating rate did not result in males providing more parental care under biparental conditions, as would be expected due to their higher perceived paternity. However, females from lines selected for high mating rate expressed lower levels of parental care than those from lines selected for low mating rate. This suggests that under biparental conditions, the costs to mating are greater for females than males in this species. However, male burying beetle parental care is known to be highly plastic, with more care provided under uniparental conditions. It is therefore unknown how the costs of mating impact parental care and offspring performance when parents care for larvae on their own, which commonly occurs in the wild. The aim of this current study therefore was to investigate the costs of a high mating rate in burying beetles under uniparental conditions, to determine whether they lead to a reduction in parental care or offspring performance. I also investigated whether these costs differ between males and females, and whether they are dependent on carcass size, a key resource for breeding for burying beetles. Individuals from lines selected for high mating rate produced fewer larvae initially, and had a lower proportion of larvae survive to dispersal, than those from lines selected for low mating rate. However, individuals from the high mating rate line spent more time providing parental care, suggesting there may be a trade-off between egg production and parental care in females. Unexpectedly, high mating rate line individuals raised larvae of greater mass than those from the low mating rate line. There was evidence for the costs of a high mating rate being greater for females than males; there was a significant effect of an interaction between the sex of the parent providing care and selection line on the proportion of time the parent spent providing direct care and on mean larval mass. There was also

a significant effect of an interaction between carcass size and selection line on the proportion of time the parent spent providing direct care and the proportion of larvae surviving to dispersal, suggesting the costs of a high mating rate are greater when breeding on a larger carcass. This study demonstrates how sex differences in the cost of mating traits, and the effect of the non-social environment on these costs, can have important consequences for parental care and offspring performance, including the coevolution of mating and parental care traits in males and females.

2.1 INTRODUCTION

A high mating rate can be beneficial for an individual's reproductive success, especially for males. In a number of species, repeated mating with the same partner can increase a male's paternity assurance by reducing sperm competition from rival males (Parker 1970; Birkhead & Pizzari 2002). Females may sometimes benefit from repeated mating by acquiring sufficient sperm to ensure all her eggs are fertilized, or by gaining direct benefits such as food provided by the male (Davies et al. 2012). However, there can also be costs of high mating rate to both males and females, and these can include energetic costs, increased risk of infection, predation and reduced time to acquire resources (Daly 1978; Rowe 1994; Chapman et al. 1995; Davies et al. 2012).The optimum mating rate is a balance between the costs and benefits of additional matings, and can differ between males and females, leading to sexual conflict (Chapman et al. 2003).

It is often predicted that there should be a trade-off, due to energetic, time or mechanistic constraints, between investment in mating traits and parental care (Trivers 1972). An example of a mechanistic constraint is the 'challenge hypothesis', which states that high testosterone, important for male-male competition and hence mating effort, decreases parental care (Wingfield et al. 1990; Schradin et al. 2009; Stiver & Alonzo 2009). Parental care, which includes behaviours such as the feeding and guarding young, is costly (Trivers 1972; Lessells 2006; Alonso-Alvarez & Velando 2012; Smiseth et al. 2012), and investing resources in the current brood is expected to reduce those available for future reproduction. An example of a trade-off between investment in mating traits and parental care comes from a study on European Starlings, *Sturnus vulgaris* (Smith 1995). Males experimentally given a second nest box spent more time singing to attract an additional female, but spent less time incubating eggs. However, there may not always be a trade-off between parental care and mating effort. For instance, in a number of fish species, females choose a mate on the basis of their parental care, and males can continue to mate while providing care (Stiver & Alonzo 2009). This makes it important to accurately test whether there is an actual trade-off between investment in these two traits, rather than measuring costs of each behaviour in isolation and then inferring

there is a trade-off. It also means that it important to have an understanding of the mechanistic cause of any trade-off between mating traits and parental care.

In addition to trade-offs between mating and parental care traits, there can also be trade-offs between investing in different elements of parental behaviour. For example, greater egg production can result in a reduction in the level of later parental care that the female provides to her offspring. Research on numerous species, including sand beetles (*Callosobruchus* spp) and birds (barn swallows *Hirundo rustica*, common terns, *Sterna hirundo*), has shown that the costs of egg production can be considerable, and can have important consequences for future parental and offspring survival (Heaney & Monaghan 1995; Monaghan & Nager 1997; Rönn et al. 2006). A recent study on the European earwig, *Forficula auricularia*, found evidence for a trade-off between egg number and pre-hatching maternal care (Koch & Meunier 2014). This trade-off was only apparent when female mass at egg laying was controlled for, demonstrating the importance of considering parent quality when investigating trade-offs in parental care. The number of nymphs hatching was found to depend on interactions between egg number, egg mass and female pre-hatching care. There was a negative association between female pre-hatching care for her first clutch and number of eggs in the second clutch when females were isolated from the nymphs of the first clutch, but not when they were allowed to interact. This suggests the presence of an additional trade-off between pre- and post-hatching care (Koch & Meunier 2014). Investment by a parent at one stage of offspring development can sometimes be masked by investment at a later stage. Egg size has a significant positive effect on the mass of burying beetle larvae in the absence of parental care, but no effect in the presence of parental care (Monteith et al. 2012).

Trade-offs between investment in different parental care traits can sometimes be masked by differences in resources available to individuals. A recent study found that in burying beetles brood size has a negative effect on larval mass (i.e there is a trade-off between offspring number and size), but that females produce offspring in greater number and of heavier mass on larger carcasses (Smiseth et al. 2014). There was a significant interaction between carcass size and brood size on larval mass, with a steeper decline in offspring mass with

increasing brood size on smaller carcasses. This indicates that resource availability can have a significant effect on trade-offs between investment in different traits (Smiseth et al. 2014). However, it is unknown whether, in burying beetles, differences in resource availability during parental care can mask the effect of costs experienced prior to parental care, such as due to high mating rate.

Male and female mating traits and parental care are closely linked; social interactions mean that trait expression in one sex will impact the phenotype and how selection acts on the opposite sex (Alonzo 2010). For instance, if a male reduces his parental care due to low paternity assurance, this can result in social (plastic change in trait expression) and coevolutionary feedback on female mating and parental care traits (Alonzo 2010). A high mating rate is an important paternity assurance trait for many species; the higher the number of times a male mates with a female, the more likely that he will father her offspring. Mating rate is genetically correlated between male and female burying beetles, suggesting that female mating rate has evolved in response to selection on males (House et al. 2008). Crosses between and within burying beetle lines artificially selected for either high or low mating rate revealed that males do not invest more in parental care when selected for higher mating rate (as would be expected due to paternity assurance being greater). In contrast, females from the high mating rate lines provided less parental care than those from the low mating rate lines, potentially due to the costs of a high mating rate for females (Head et al. 2014). However, while this study focused on biparental care, uniparental care (especially by females) also occurs frequently in the wild (Eggert & Müller 1997). Male burying beetle parental care is known to be highly plastic to the presence of a female partner, with little care provided under biparental conditions but similar levels to females under uniparental conditions (Smiseth et al. 2005). Therefore, costs to males of a high mating rate may be more visible under uniparental conditions than biparental. It is therefore unknown whether, under uniparental care, the cost of mating differs between male and female burying beetles, and how this impacts parental care and larval performance. These studies above demonstrate the importance of considering the coevolution between male and female mating and parental care traits when making predictions regarding their response to selection (Alonzo 2010).

In order to fully understand how male and female mating traits and parental care coevolve, it is important to have a detailed understanding of the costs to both sexes of mating and different stages of parental care. It is also crucial to have knowledge of trade-offs between different stages of parental care, and how these costs and trade-offs may differ depending on the resources available and the quality of the individual (such as their size). Burying beetles, *Nicrophorus vespilloides*, are an ideal species in which to investigate questions regarding the costs of a high mating rate and trade-offs between different aspects of parental care. Parental care is varied in this species, and has a large impact on offspring survival and fitness (Eggert & Müller 1997; Scott 1998). Adults process and bury a small vertebrate carcass such as a mouse in the soil, which becomes a food source for their larvae (Eggert & Müller 1997; Scott 1998). Care can be provided by either parent alone (uniparental care) or together (biparental care) (Royle et al. 2013). There is often strong male-male and female-female competition over the use of vertebrate carcasses to breed on, as they are an ephemeral resource. Individual size is an important determinant of burying beetle competitive ability (Otronen 1988), and parental care has a positive effect on larval growth (Eggert et al. 1998). Carcass size is also a major determinant of larval growth (Bartlett & Ashworth 1988; Hopwood et al. 2014). Adult burying beetle size is determined by larval growth, as after eclosion adult beetles can increases in mass but not size.

Following on from Head et al (2014), the aim of this present study was to investigate in more detail the relationship between mating traits and parental care behaviours, using the same burying beetle lines artificially selected for high and low mating rate as a model system. Specifically, do any costs of a high mating rate, and its impact on parental care, differ between the sexes when caring alone? Is there a trade-off between investment in mating and investment in parental care? How do any potential costs of a high mating rate impact on trade-offs in females between investment in egg production vs. later parental care? To address these questions, within-line matings were set up in either poor or good resource conditions (small or large mouse carcass), and mating rate, egg production, parental care and larval performance were recorded. One parent, male or female, was removed prior to larvae hatching leading to uniparental care. This study differs from Head et al (2014) by focusing on

potential costs of mating to males and females under uniparental care as opposed to biparental care. A potential issue of using a within-line design for assessing the impact of high or low mating rate on parental care is that the behaviour of the focal parent may be influenced by the behaviour of their partner, and individuals were always paired with another from the same line. Including both within and between-line crosses in the experimental design would have allowed the influence of partner behaviour to be eliminated. However, as the partner was removed prior to larvae hatching, and the number of larvae was standardised, it is likely that partner behaviour (other than their influence on the pair's mating rate) did not have a large impact on the focal individual's parental care during the experiment.

An additional aim of this study was to investigate the effect of resource availability (carcass size, an important element of the non-social environment), on the costs of a high mating rate. It may be that high mating rate line individuals are unable to fully utilise the extra resources of a large carcass, making the costs of a high mating rate more visible. In contrast, on small carcasses, high mating rate line individuals may be less able to recuperate costs by consuming the carcass themselves, and larval demand for care may be increased. Therefore, the costs of a high mating rate could potentially be greater on either small or large carcasses.

For females, costs of a high mating rate could result in a reduction in the number of eggs laid, parental care or larval performance (larval mass, survival to dispersal). There could also be costs of a high mating rate for males, leading to a reduction in their parental care or larval performance. Costs of a high mating rate for males and females could be due to energetic costs of mating or injury (especially for females in resisting males), leaving the individual with less resources to invest in parental care behaviours (Daly 1978; Rowe 1994; House et al. 2008). I predict that high mating rate line females will show a trade-off between egg production and parental care. If high mating rate line females produce fewer eggs, they may be able to maintain similar levels of parental care, and larval performance may not be significantly reduced. If the costs of a higher mating rate results in lower levels of parental care but maintained brood size, I predict high mating rate line individuals will produce larvae of lower mass

and have fewer larvae surviving to dispersal than individuals from the low mating rate line. Following from Head et al (2014), I predict that costs of a higher mating rate will be greater for females than for males; this would be revealed by a significant interaction between selection line (mating rate) and the sex of the parent providing care on measures of parental care or larval performance. I also predict that any costs of a high mating rate will be dependent upon carcass size due to its importance for both parent and offspring fitness. However, as discussed above, it is difficult to predict whether costs of a high mating rate will be relatively greater on large or small carcasses. Evidence for this would be a significant interaction between carcass size and selection line (mating rate) on one of the measures of parental care or larval performance.

2.2 METHODS

ORIGIN AND MAINTENANCE OF BEETLE SELECTION LINES

180 Burying beetles (*Nicrophorus vespilloides)* were collected from Devichoys wood (N 50º11'47''E-5º7'23''), UK, in July 2010 and maintained in the lab for 3 generations. Beetles were bred by placing a virgin male and female non-sibling pair into a transparent plastic breeding box (17x12x6 cm) filled with 2cm of moist soil and a moist 15-25g mouse carcass (Livefoods Direct, Sheffield). Once larvae had dispersed from the carcass, approximately 8 days later, they were placed in individual transparent plastic pots (7x7x4 cm) filled with 2cm of moist soil. Larvae were considered to have dispersed when at least two larvae in the brood were present away from the carcass. After eclosion (around 18 days post larval dispersal), adult beetles were fed two decapitated mealworms (*Tenebrio moliter*) twice weekly. Adult beetles were bred after reaching reproductive maturity at around 14 days post-eclosion**.**

After three generations in the lab, selection lines for high and low mating rate were established. Selection lines were generated by carrying out mating trials (see below), selecting the 20% of individuals with the highest or lowest mating rate to produce the High and Low lines respectively. After one generation of selection each selection line was split into two replicates (H1, H2, L1, L2). For further details on the origin and maintenance of selection lines see Head et al.

2014, Carter et al. 2015; Hopwood et al. 2016. This study was conducted on generation 24 of the selection lines.

EXPERIMENTAL DESIGN

The experiment started when the beetles were between 14-21 days post eclosion, and was conducted in the dark in a temperature controlled room maintained at 20(+/- 1)°C. Adult beetle size was calculated by measuring the width of the pronotum to 0.01mm using digital callipers. Adult beetle mass was measured to 0.1mg using an Ohaus Explorer digital balance.

Mating trials were conducted for non-sibling male-female pairs, with both individuals coming from the same selection line and replicate. Trials started once both individuals had been placed on a 8.5cm diameter petri dish lined with filter paper, and the number of matings during an hour was recorded. Later on the same day as the mating trial, at approximately 18h, each pair was transferred to a transparent breeding box (17x12x6 cm), filled with 2cm of moist soil which contained either a small (mean 10.3g +/- 0.14SE) or large (mean 24.5g +/- 0.21SE) mouse carcass. 65 hours later the mouse carcass and one adult beetle from each pair were moved to a new breeding box, and the other adult disposed of. A small amount of rotting meat was placed in the original breeding box to attract larvae after hatching and to prevent them from starving. Breeding boxes were checked for the presence of larvae every 8 hours. Once larvae had hatched, 20 from each family were transferred to the new breeding box containing the carcass and their biological parent. Families with fewer than 20 larvae were supplemented with larvae of similar age from other families from the same selection line.

Parental care observations started four hours after larvae were placed in the new breeding box, or the next day if larvae were transferred late in the afternoon. Four observations were carried out each day (at 8,11,14 and 17h) and ended at the point that the larvae had dispersed. For each observation the parent was recorded as either providing direct (feeding/ touching larvae), indirect (present on carcass), or no care (away from carcass) at this time point. Larval dispersal was counted as the point that at least two larvae were present away from the carcass. At dispersal, larvae were rinsed with water, dried with

tissue paper, and were individually weighed to 0.1mg using a Ohaus Explorer digital balance. The initial sample size across both sexes for each selection line were as follows; 63 H1, 57 H2, 75 L1, 58 L2.

DATA ANALYSIS

Statistics were carried out using R version 3.3.0 (R Core Team 2016), using the package lme4 to produce generalized linear mixed models. The response variables analysed were number of larvae initially produced, proportion of observations in which the parent was observed caring (either direct care only, indirect care only, or total (direct plus indirect) care), proportion of larvae surviving to dispersal, time taken for larvae to disperse, average larval mass and coefficient of variation of larval mass. Proportion of time providing direct care, proportion of time providing indirect care and proportion of larvae surviving to dispersal were analysed with a binomial error structure with a logit link, using the Laplace approximation.

For the number of larvae initially produced, the explanatory variables were size of carcass (large or small), size of the female, selection line (high or low), an interaction between carcass size and selection line and an interaction between the size of the female and size of carcass. The size of the male was not included in this model as there was no a priori reason to expect it to impact the number of eggs the female laid, and it is best to avoid adding additional terms to models when the sample size is not especially large.

The explanatory variables for the parental care models (proportion of observations direct care, indirect care or total care) were size of the carcass, size of the parent providing care, selection line, sex of parent providing care, an interaction between sex of parent providing care and selection line, and an interaction between carcass size and selection line.

For the measures of larval performance (proportion of larvae surviving to dispersal, dispersal time, average larval mass and coefficient of variation of larval mass), the explanatory variables were the same as the parental care models with the addition of proportion of observations in which care (direct plus indirect) was recorded.

Each model included selection line replicate (H1, H2, L1, L2) as a random effect allowed to vary in intercept only, and were fitted using the maximum likelihood method. All binomial models also included an observation level random effect to reduce overdispersion (Harrison 2015). Models were tested using backward step elimination, starting with interaction terms, until only significant terms ($P < 0.05$) remained in the model (Bolker et al. 2009; Crawley 2013). Once found, the linear mixed minimal models were refitted using the REML criterion, and the significance of their random effects tested using the package LmerTest. Binomial linear mixed models cannot be refitted using REML, nor the significance of their random effects tested using this package. The stated P value of non-significant variables is that at the point of their removal from the model. The P value for significant variables is that for their removal from the minimal model.

To investigate whether the differences between selection lines were due to the effects of mating rate *per se* (rather than a consequence of divergence between lines due to selection), each of the above models were refitted using the mating rate of each pair instead of selection line (including for interactions). No random effects were included in these models, and binomial models were converted to quasibinomial to reduce overdispersion. Minimal models were selected, as above, by backwards step elimination. These results are given in the appendix, except where there was a significant difference between the selection line and mating rate models.

2.3 RESULTS

EFFECT OF SELECTION REGIME, FEMALE SIZE AND CARCASS SIZE ON NUMBER OF LARVAE PRODUCED

The interaction between carcass size and selection line was not significant, nor was the interaction between carcass size and female size, on the number of larvae initially produced (both $P > 0.29$). Low mating rate line pairs produced significantly more larvae than high mating rate line pairs ($\chi^2 = 9.05$, $df = 1$, $P < 0.003$) (Fig 1). The size of the female was marginally non-significant, with larger females having a tendency to produce more larvae ($\chi^2 = 2.97$, $df = 1$, $P =$

0.085). There was no significant effect of carcass size on the number of larvae produced (χ^2 = 0.95, *df* = 1, *P* = 0.331). (Table 1)

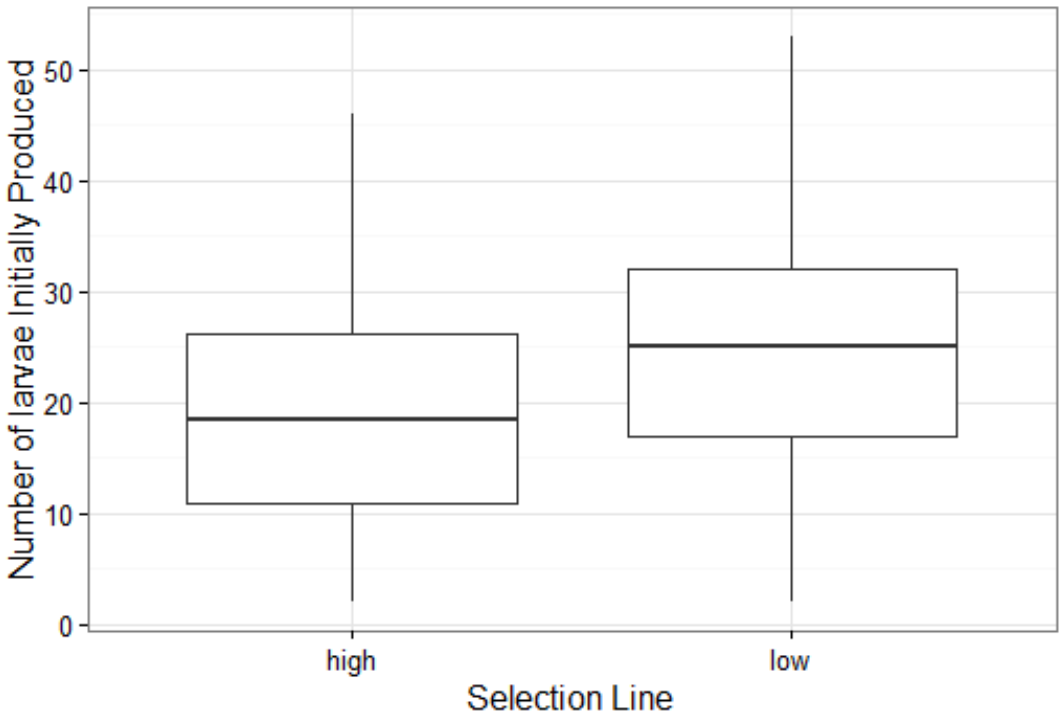

Fig 1. *Low mating rate line pairs produced significantly more larvae initially than high mating rate line pairs. Mean number of larvae initially produced (+/-standard error); high mating rate line = 19.26(0.9), low mating rate line = 24.25(0.93).*

EFFECT OF SELECTION REGIME, SIZE AND SEX OF PARENT PROVIDING CARE AND CARCASS SIZE ON PARENTAL CARE

The interaction between the sex of the parent providing care and the selection line was significant (χ^2 = 4.64, *df* = 1, *P* = 0.031), as was the interaction between carcass size and selection line (χ^2 = 7.52, *df* = 1, *P* = 0.006), on the proportion of time that the parent spent providing direct care (Fig 2). On average, low mating rate line males spent a lower proportion of time providing direct care than females, while high mating rate line males spent more time providing care than females (Table 1). Beetles from the low mating rate line showed a decrease in the average proportion of time spent providing direct care on small carcasses compared to large carcasses, whereas high mating rate line individuals showed an increase (Table 1). However, neither of these interactions was significant (or any of the terms as a main effect) in the alternative model using mating rate instead of selection line (see appendix). There was no significant effect of the size of the parent providing care on the proportion of time providing direct care (χ^2 = 1.04, *df* = 1, *P* = 0.307). (Table 1)

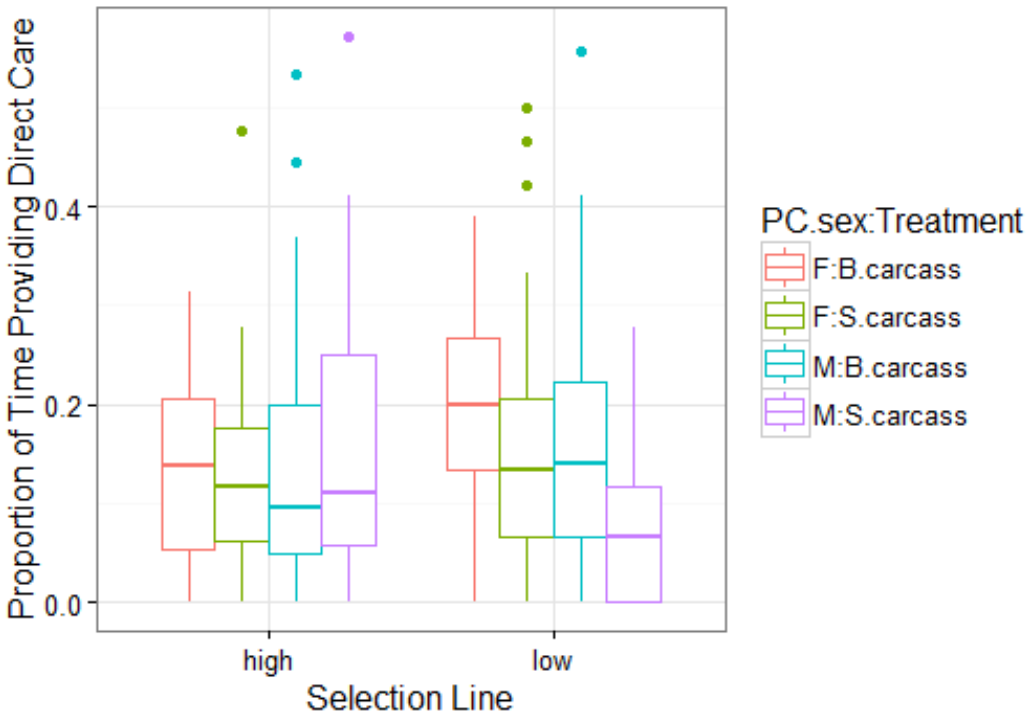

Fig 2. *The interaction between carcass size and selection line, and between the sex of parent providing care and selection line, had a significant effect on the proportion of time that the parent provided direct care. PC.sex- sex of parent providing care; Treatment- size of carcass (big (B) or small (S)). Mean proportion of time providing direct care {selection line:PC.sex:Treatment} (+/-standard error); high:F:B.carcass = 0.14(0.02), high:F:S.carcass = 0.14(0.02), high:M:B.carcass = 0.14(0.02), high:M:S.carcass = 0.16(0.02), low:F:B.carcass =0.19(0.02), low:F:S.carcass = 0.16(0.02), low:M:B.carcass = 0.16(0.02), low:M.S.carcass = 0.08(0.01).*

The interaction between the sex of the parent providing care and the selection line was not significant, nor was the interaction between carcass size and selection line, on the proportion of time that the parent spent providing indirect care (both $P > 0.34$). There was a significant effect of selection regime, with low mating rate line individuals spending less time providing indirect care ($\chi^2 = 5.54$, $df = 1$, $P = 0.019$) (Fig 3). Males provided significantly less indirect care than females ($\chi^2 = 29.49$, $df = 1$, $P < 0.0001$) (Fig 3). The effect of the size of the parent providing care was only marginally non-significant, with larger parents spending less time providing indirect care ($\chi^2 = 3.64$, $df = 1$, $P = 0.056$). The size of the carcass did not have a significant effect on indirect care ($\chi^2 = 0.68$, $df = 1$, $P = 0.41$) (Table 1). Although the effect of selection regime was significant in this model, mating rate did not have significant effect on the time spent providing indirect care in the alternative model (see appendix).

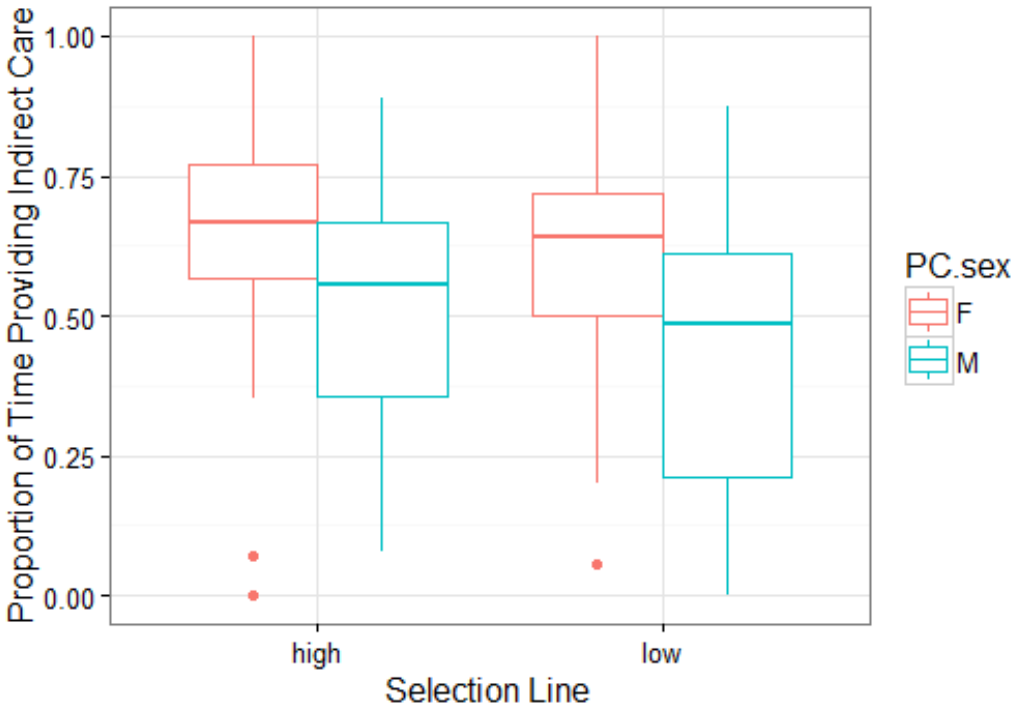

Fig 3. *Low mating rate line individuals spent a significantly lower proportion of time providing indirect care than high mating rate line individuals, and males spent a significantly lower proportion of time providing indirect care than females. PC.sex- sex of parent providing care. Mean proportion of time providing indirect care {selection line,:PC.sex}(+/-standard error); high:F = 0.66(0.03), high:M = 0.52(0.03), low:F = 0.6(0.02), low:M = 0.44(0.03).*

The interaction between the sex of the parent providing care and the selection line was not significant, nor was the interaction between carcass size and selection line, on the total proportion of time that the parent spent providing care (direct plus indirect) (both $P > 0.16$). The size of the parent had a significant negative effect on the proportion of time they spent providing care ($\chi^2 = 5.48$, $df = 1$, $P = 0.019$), and males spent significantly less time caring than females ($\chi^2 = 29.6$, $df = 1$, $P < 0.0001$) (Fig 4). The effect of selection regime was only marginally non-significant, with low mating rate line individuals providing less care than high mating rate line individuals ($\chi^2 = 3.76$, $df = 1$, $P = 0.053$). There was no significant effect of carcass size on time spent caring ($\chi^2 = 2.42$, $df = 1$, $P = 0.12$). (Table 1)

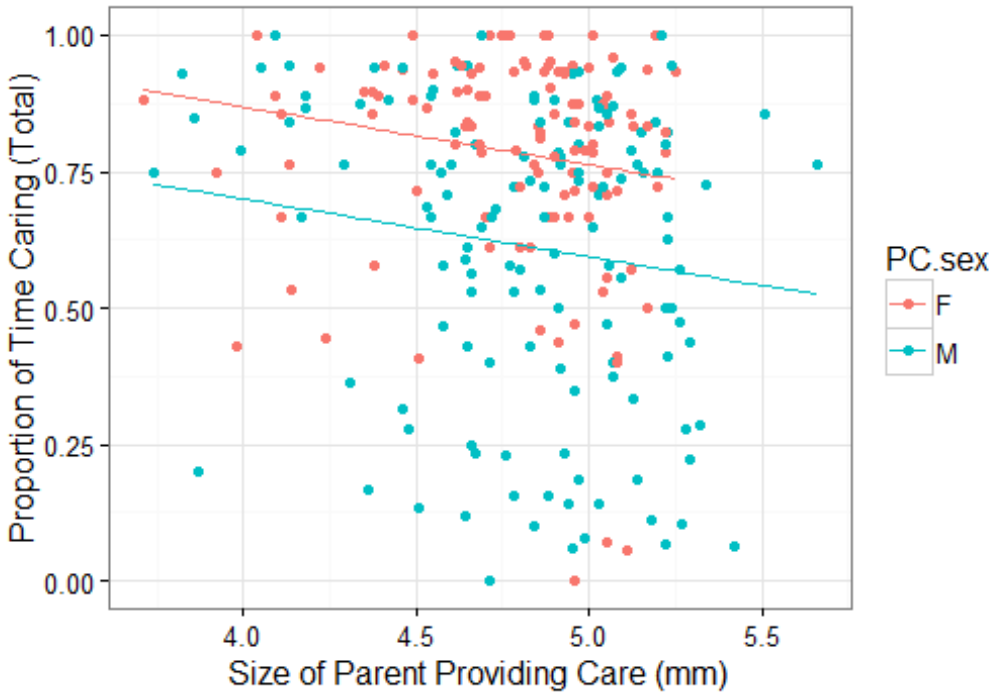

Fig 4. *Larger individuals spent a significantly lower proportion of time providing care in total (direct plus indirect care), and males spent significantly lower proportion of time caring than females. PC.sex- sex of parent providing care. Regression lines are those given by the minimal model.*

EFFECT OF SELECTION REGIME, SIZE AND SEX OF PARENT PROVIDING CARE, CARCASS SIZE AND PARENTAL CARE ON LARVAL PERFORMANCE

The interaction between carcass size and selection regime had a significant effect on the proportion of larvae surviving to dispersal ($\chi^2 = 4.33$, *df* = 1, *P* = 0.037) (Fig 5). Both lines showed a decrease in average number of larvae surviving to dispersal on small compared to large carcasses, but this effect was greater for the low mating rate line (Table 1). In the alternative model, the interaction between mating rate and carcass size was not significant (see appendix). The interaction between the sex of the parent providing care and the selection regime was not significant ($\chi^2 = 1.16$, *df* = 1, *P* = 0.282). Females raised significantly more larvae to dispersal than males ($\chi^2 = 6.52$, *df* = 1, *P* = 0.011). Individuals that spent a greater proportion of time providing care also had significantly more larvae survive to dispersal ($\chi^2 = 7.98$, *df* = 1, *P* = 0.005) (Fig 5). The size of the parent providing care did not have a significant effect on the proportion of surviving larvae ($\chi^2 = 1.65$, *df* = 1, *P* = 0.199). (Table 1)

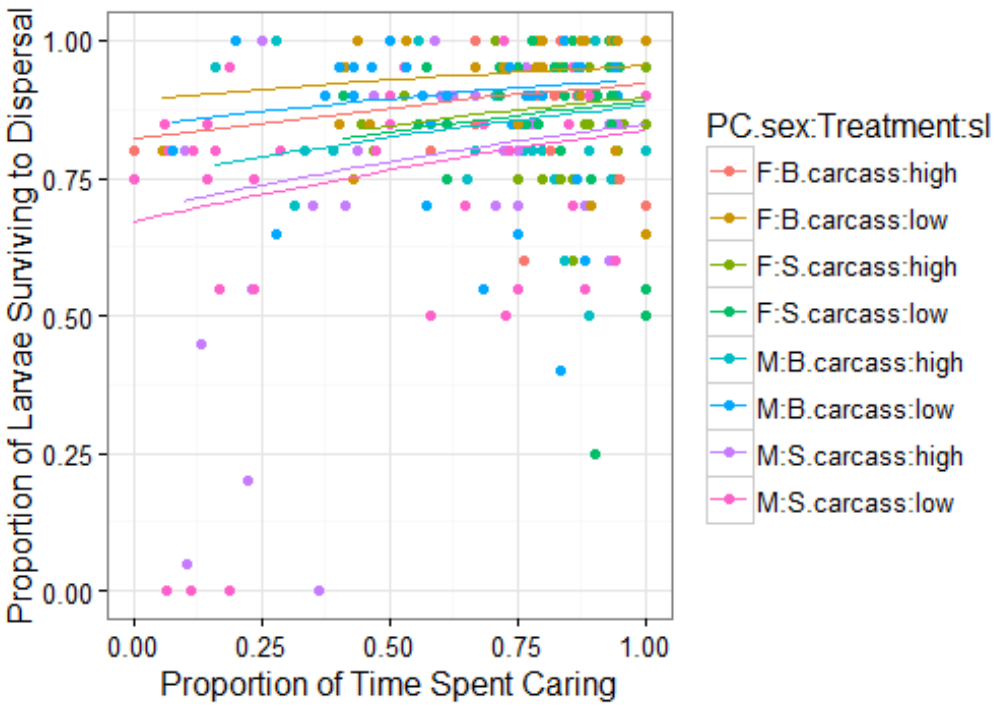

Fig 5. *The interaction between carcass size and selection line had a significant effect on the proportion of larvae surviving to dispersal. The proportion of time that the parent spent caring in total (direct plus indirect care) had a significant positive effect on proportion of larvae surviving. Males had a significantly lower proportion of larvae surviving to dispersal than females. PC.sex- sex of parent providing care; Treatment- size of carcass (big (B) or small (S)); sl- selection line (high or low mating rate). Regression lines are those given by the minimal model.*

The interaction between the sex of the parent providing care and selection line was not significant, nor was the interaction between carcass size and selection line, on the time taken for larvae to disperse (both $P > 0.24$). Larvae cared for by males took significantly longer to disperse than those cared for by females ($\chi^2 = 4.05$, $df = 1$, $P = 0.044$). The larvae of parents that spent a greater proportion of time caring took significantly longer to disperse ($\chi^2 = 7.12$, $df = 1$, $P = 0.008$) (Fig 6). There was no significant effect of the size of the parent providing care ($\chi^2 = 0.01$, $df = 1$, $P = 0.943$), carcass size ($\chi^2 = 0.39$, $df = 1$, $P = 0.53$) or selection regime ($\chi^2 = 0.9$, $df = 1$, $P = 0.342$) on the time taken for larvae to disperse. (Table 1)

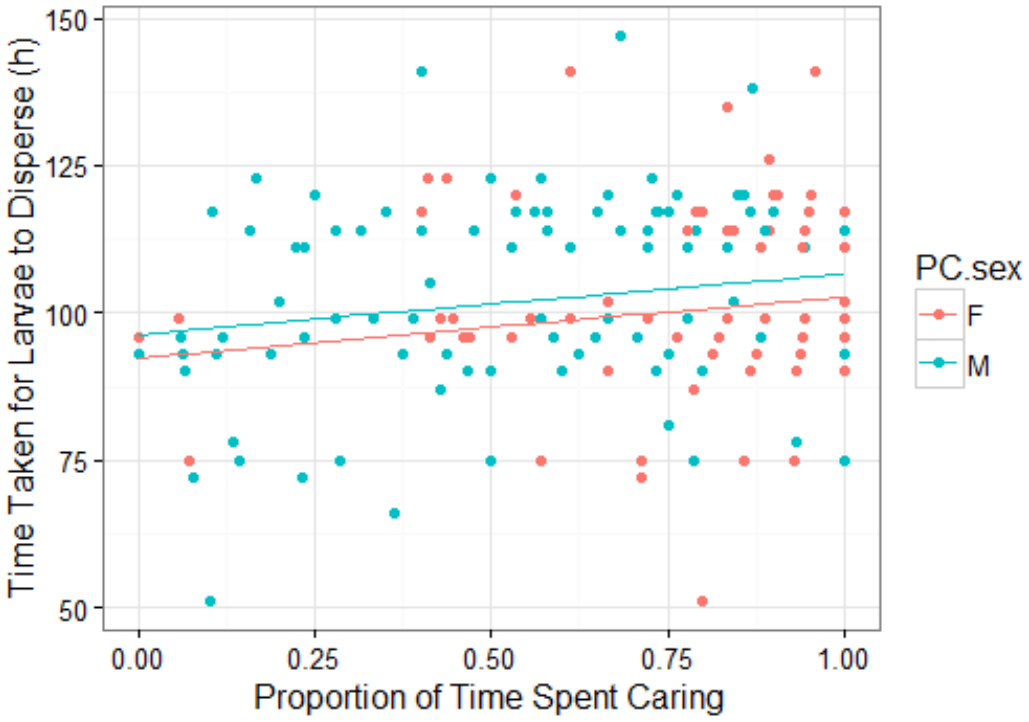

Fig 6. *Larvae raised by males took significantly longer to disperse than those raised by females. The offspring of parents who spent a greater proportion of time providing care took significantly longer to disperse. PC.sex- sex of parent providing care. Regression lines are those given by the minimal model.*

The interaction between selection line and the sex of the parent providing care had a significant effect on mean larval mass ($\chi^2 = 4.27$, *df* = 1, *P* = 0.039). For the low mating rate line there was a slight increase in average larval mass under male care compared to female care. In contrast, for the high mating rate line there was a decrease in average larval mass under male compared to female care (Table 1, Fig 7). The interaction between mating rate and the sex of the parent providing care was not significant in the alternative model (see appendix). For selection regime as a main effect, high mating rate line larvae were heavier on average than low mating rate line larvae (Table 1, Fig 7). This was supported by the alternative model, in which mating rate had a significant positive effect on offspring mass (see appendix). The sex of the parent providing care was not significant as a main effect in the alternative model (see appendix). The interaction between carcass size and selection line was not significant (*P* > 0.98). Mean larval mass was significantly decreased on smaller carcasses ($\chi^2 = 89.93$, *df* = 1, *P* < 0.0001) (Fig 7). Larger parents providing care produced heavier larvae ($\chi^2 = 5.19$, *df* = 1, *P* = 0.023) (Fig 8), as did those that spent a greater proportion of time caring ($\chi^2 = 43.97$, *df* = 1, *P* < 0.0001) (Fig 9). (Table 1)

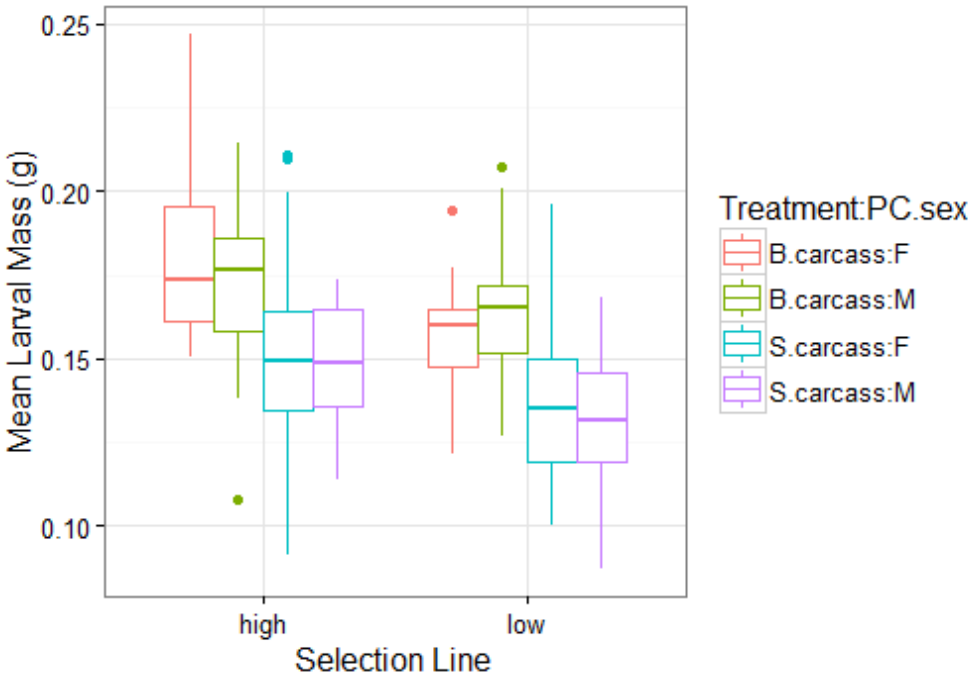

Fig 7. *The interaction between the sex of the parent providing care and selection line had a significant effect on mean larval mass. Larval mass was significantly reduced on smaller carcasses. PC.sex- sex of parent providing care; Treatment- size of carcass (big (B) or small (S)). Mean of mean larval mass(g) {selection line:Treatment:PC.sex} (+/-standard error); high:B.carcass:F = 0.18(0.005), high:B.carcass:M = 0.17(0.004), high:S.carcass:F = 0.15(0.005), high:S.carcass:M = 0.15(0.003), low:B.carcass:F = 0.16(0.003), low:B.carcass:M = 0.16(0.003), low:S.carcass:F = 0.14(0.004), low:S.carcass:M = 0.13(0.004).*

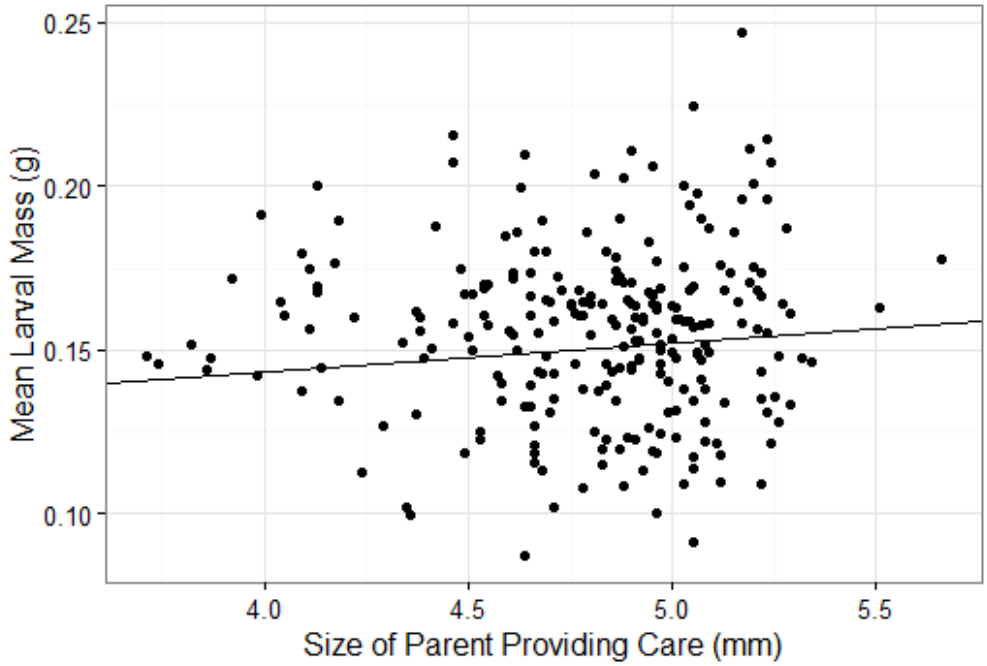

Fig 8. *Larger parents providing care produced larvae of significantly greater mass. Regression line is that given by the minimal model.*

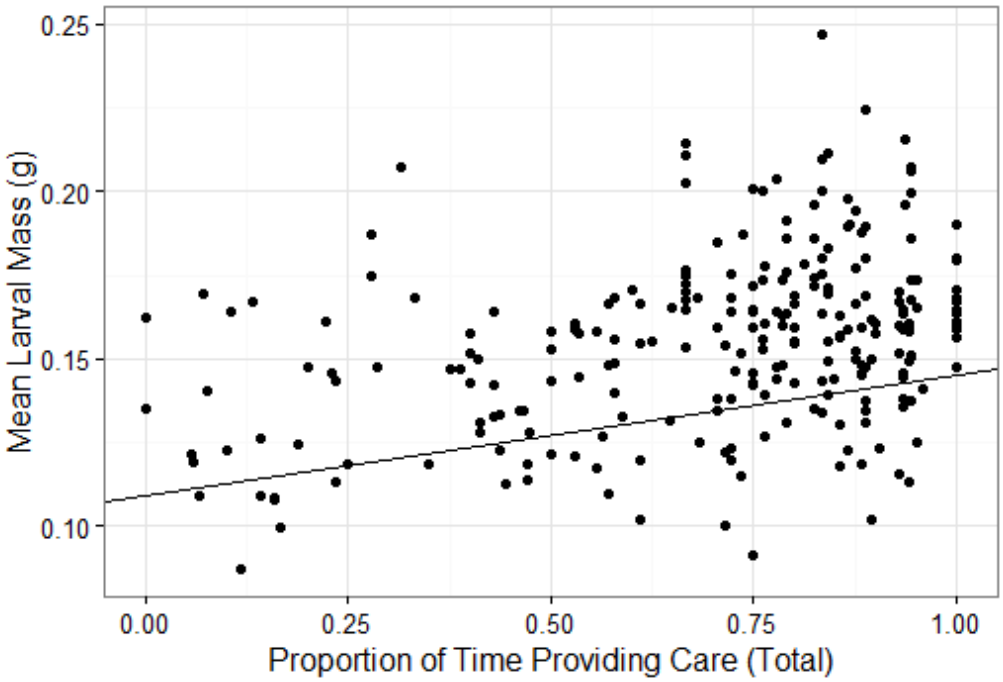

Fig 9. *Parents that spent a greater proportion of time caring (direct plus indirect care) produced larvae of significantly greater mass. Regression line is that given by the minimal model.*

The interaction between selection line and sex of parent providing care was not significant, nor was the interaction between carcass size and selection line, on the coefficient of variation of larval mass (both $P > 0.66$). Variation in larval mass was significantly increased on small carcasses ($\chi^2 = 29.74$, $df = 1$, $P < 0.0001$), and decreased by greater proportion of time spent caring by the parent ($\chi^2 = 11.06$, $df = 1$, $P = 0.001$) (Fig 10). There was no significant effect of the selection line ($\chi^2 = 0.39$, $df = 1$, $P = 0.534$), the sex of the parent providing care ($\chi^2 = 0.51$, $df = 1$, $P = 0.476$), or of the size of the parent providing care ($\chi^2 = 0.9$, $df = 1$, $P = 0.344$) on the coefficient of variation of larval mass. (Table 1)

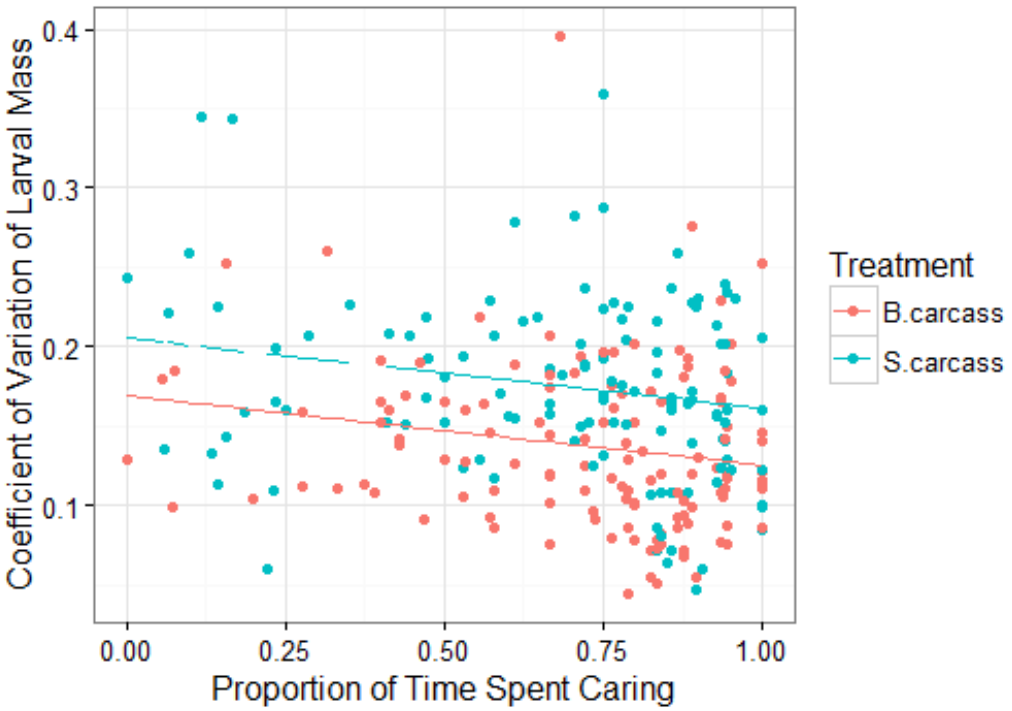

Fig 10. *The coefficient of variation of larval mass was significantly greater for larvae raised on small carcasses than those raised on large carcasses. The proportion of time that the parent provided care for (direct plus indirect care) had a significant negative effect on the coefficient of variation of larval mass. Treatment- size of carcass (big (B) or small (S)). Regression lines are those given by the minimal model.*

Response Variable		**Terms in Minimal Model**	**Model Coefficients** (+/- SE)	**t/z value/ Chi.sq**	**d.f.**	**Pr(>|t|)/ Pr(>|z|)/ P.value**
Number of Larvae Initially Produced	Fixed Effects	Intercept	19.26 (0.94)	20.52	251	< 0.0001
		Selection Line (Low)	4.99 (1.29)	3.85	251	0.0001
	Random Effects	Selection Line Rep		6.82×10^{-13}	1	1
Proportion of Time Providing Direct Care	Fixed Effects	Intercept	-2.04 (0.17)	-12.33	**	< 0.0001
		Carcass Size (Small)	0.13 (0.18)	0.73	**	0.465
		Selection Line (Low)	0.63 (0.22)	2.91	**	0.004
		PC.Sex (Male)	0.05 (0.18)	0.3	**	0.765
		Selection Line (Low): PC.Sex (Male)	-0.53 (0.25)	-2.16	**	0.031
		Carcass Size (Small): Selection Line (Low)	-0.68 (0.25)	-2.74	**	0.006
	Random Effects	Selection Line Rep		*	*	*
Proportion of Time Providing Indirect Care	Fixed Effects	Intercept	0.76 (0.11)	6.67	**	< 0.0001
		Selection Line (Low)	-0.32 (0.13)	-2.53	**	0.012
		PC.Sex (Male)	-0.7 (0.13)	-5.55	**	< 0.0001
	Random Effects	Selection Line Rep		*	*	*
Proportion of Time Providing Care (Total)	Fixed Effects	Intercept	1.29 (0.22)	6	182.26	< 0.0001
		Size of Parent Providing Care	-0.11 (0.04)	-2.36	188.54	0.019
		PC.Sex (Male)	-0.17 (0.03)	-5.59	247.3	< 0.0001
	Random Effects	Selection Line Rep		0.44	1	0.5
Proportion of Larvae Surviving to Dispersal	Fixed Effects	Intercept	1.52 (0.34)	4.51	**	< 0.0001
		Carcass Size	-0.28 (0.23)	-1.24	**	0.217

Proportion of Larvae Surviving to Dispersal (continued)		Selection Line (Low)	0.59 (0.25)	2.41	**	0.016
		PC.Sex (Male)	-0.44 (0.17)	-2.56	**	0.01
		Proportion of Time Caring	0.93 (0.33)	2.84	*	0.005
		Carcass Size (Small): Selection Line (Low)	-0.67 (0.32)	-2.09	*	0.037
	Random Effects	Selection Line Rep		*	*	*
Time taken for Larvae to Disperse	Fixed Effects	Intercept	92.41 (4.57)	20.22	10.84	< 0.0001
		PC.Sex (Male)	3.93 (1.95)	2.02	247.12	0.045
		Proportion of Time Caring	10.3 (3.82)	2.69	247.67	0.008
	Random Effects	Selection Line Rep		24.4	1	< 0.0001
Mean Larval Mass	Fixed Effects	Intercept	0.11 (0.02)	5.58	174.5	< 0.0001
		Carcass Size (Small)	-0.03 (0.002)	-10.39	240.3	< 0.0001
		Size of Parent Providing Care	0.01 (0.004)	2.26	237.9	0.025
		Selection Line (Low)	-0.02 (0.01)	-3.07	2.810	0.06
		PC.Sex (Male)	-0.001 (0.004)	-0.26	240	0.799
		Proportion of Time Caring	0.04 (0.01)	6.83	240.8	< 0.0001
		Selection Line (Low): PC.Sex (Male)	0.01 (0.005)	2.06	240	0.041
	Random Effects	Selection Line Rep		5.52	1	0.02
Coefficient of Variation Larval Mass	Fixed Effects	Intercept	0.17 (0.01)	11.53	10.17	< 0.0001
		Carcass Size (Small)	0.04 (0.01)	5.6	242.25	< 0.0001
		Proportion of Time Caring	-0.04 (0.01)	-3.35	242.77	0.001
	Random Effects	Selection Line Rep		23.2	1	< 0.0001

Table 1. *Analysis of number of larvae initially produced, proportion of time spent providing parental care (direct, indirect or both) and larval performance traits. Minimal models were refitted using the REML method to give the coefficients of the fixed effects. For the LMMs, t values for the coefficients of the fixed effects and their degrees of freedom (using the* Satterthwaite approximation) are given. *The chisq value and significance of the random effects is also presented. Proportion of time providing direct care, proportion of time providing indirect care and proportion of larvae dispersing were analysed using binomial generalized linear mixed models, which included an observation level random effect to reduce overdispersion in addition to selection line rep. These models could not be fitted using the REML method, and the significance of their random effects could not be tested. The Z values of the GLMM fixed effect coefficients are given. PC.Sex is the sex of the parent providing care. Proportion of Time Caring is the proportion of time the parent spent providing care in total (direct plus indirect care).*
** The significance of the random effect, Selection Line Rep, could not be tested in the GLMMs.*
***The degrees of freedom for the z test for each fixed effect in the GLMMs could not be calculated by the statistics package used.*

2.4 DISCUSSION

In order to understand observed patterns and the co-evolution of mating traits and parental care, it is important to have information regarding the costs of each. The aim of this study was to investigate in burying beetles whether there are costs of a high mating rate, and whether these costs differ between males and females. Predictions were supported of there being a cost of a high mating rate on offspring production and survival; females from lines selected for high mating rate produced fewer larvae initially, and individuals with higher mating rates had a tendency to have fewer larvae surviving to dispersal. High mating rate line individuals produced larvae of greater mass, supporting previous results which have suggested a trade-off between larval mass and number (Smiseth et al. 2014). Unexpectedly, high mating rate line individuals spent more time providing care than those from the low mating rate line, suggesting a potential trade-off between egg-production and parental care. However, it is possible that the care provided by high mating rate line individuals is of lower quality, increasing the proportion of time that they need to spend on the activity. Following predictions, there was evidence for the costs of mating being greater for females than males. These results also provide evidence for the costs of a high mating rate varying under different (non-social) environmental conditions (breeding on different sized carcasses). The cost of a high mating rate was relatively greater when breeding on large carcasses than small, suggesting high mating rate line individuals are unable to take full advantage of the extra

resources of a large carcass. However, none of the interactions suggesting a higher cost of mating to females than males, or higher costs when breeding on a larger carcass, were significant in the alternative model using mating rate instead of selection line. This suggests that these differences between selection lines may be due to the result of long term selection and divergence between lines, rather than short term costs to individuals of mating.

COSTS OF A HIGH MATING RATE ON PARENTAL CARE AND LARVAL PERFORMANCE

Selection on mating rate had a significant effect on the number of larvae initially produced. Pairs from lines selected for high mating rate produced significantly fewer offspring, suggesting that there is a cost of a high mating rate. That this is due to the cost of mating itself is further supported by the alternative model, using mating rate instead of selection line, which produced similar results. This may be due to costs of high mating rate making females physically unable to lay more eggs. Alternatively, the costs could mean that the parents are unable to care for as many larvae, so the female adaptively reduces the number of eggs laid (Müller et al. 1990). There is also the possibility that a high mating rate and disturbance from the male may result in the female having less time to lay eggs before caring for the first hatching. As expected, larger females had a tendency (significant in the alternative model) to produce more larvae, as found by previous studies (Bartlett & Ashworth 1988).

Beetles from the high mating rate line had a marginally non-significant tendency to spend a greater proportion of time providing larvae with care than those from the low mating rate line. This difference is due to high mating rate line individuals spending significantly more time providing indirect care than those from the low mating rate line. This suggests that there is a trade-off between egg production and parental care, with high mating rate line pairs compensating for producing fewer offspring by providing more care. Trade-offs between investing in egg production and parental care have been found in other species, such as European earwigs (see introduction) (Koch & Meunier 2014). However, there is the possibility that high mating rate line individuals need to spend more time providing parental care as a result of their care being of lower quality due to the costs of mating. The results of this study contrast with those of Head et

al. (2014), which found that high mating rate line females cared for offspring for a shorter duration of time than those from the low mating rate line. These differences in results could be explained by the fact that Head et al. (2014) investigated the duration of care under biparental conditions, whereas this study measured the frequency of care under uniparental conditions. Male burying beetle care is known to be plastic in relation to the social environment, with males providing more care under uniparental conditions (Smiseth et al. 2005). In the alternative model, mating rate did not have a significant effect on the total proportion of time a parent spent providing care, suggesting that the difference between selection lines may not be due to differences in mating rate *per se*. Males spent significantly less time providing indirect care, and care in total, than females. Previous studies have found that male burying beetles provide less direct care than females under biparental conditions (Smiseth & Moore 2004; Smiseth et al. 2005). Larger individuals spent a significantly smaller proportion of time caring than smaller individuals, which could potentially be due to larger individuals being more efficient in their parental care (Steiger 2013).

There was also evidence for the costs of a high mating rate impacting larval performance. Mating rate had a marginally non-significant negative effect on the proportion of larvae surviving to dispersal. This follows Head et al. (2014) results, who found that the offspring of females from the high mating rate line had lower performance. Given that high mating rate line individuals have a tendency to spend more time providing care, this suggests that the costs of a high mating rate result in lower quality of care, or that increased stress from high mating rate may result in increased cannibalism of larvae (Bartlett 1987). However, high mating rate had a positive effect on mean larval mass. This may be due to lower competition between larvae as fewer of them survive until dispersal. These results therefore follow those of previous studies which have identified a trade-off between larval number and mass (Smiseth et al. 2014).

Other results for larval performance matched those expected from previous studies. On smaller carcasses, the proportion of larvae surviving to dispersal and mean larval mass were significantly lower than on larger carcasses, as was expected from previous studies (Bartlett & Ashworth 1988; Smiseth et al. 2014; Hopwood et al. 2014). In addition, parental care had a significant positive effect

on larval survival and mean larval mass, as identified by previous studies (Eggert et al. 1998). Broods raised by males had significantly fewer larvae surviving to dispersal than those raised by females. The size of the parent proving care had a significant positive effect on mean larval mass. This result is consistent with previous studies, which found that larger females produce heavier larvae, potentially due to them being more efficient at provisioning offspring (Steiger 2013).

Selection regime did not have a significant effect on either dispersal time or the coefficient of variation of larval mass, suggesting that these traits are not affected by any costs of a high mating rate. The larvae of parents that spent a greater proportion of time caring took longer to disperse, which may be due to the end of care being a signal for dispersal. Larvae raised by males took longer to disperse than those raised by females, which is surprising given that males tend to provide less care. However, this may be explained by males and females differing in their relative investment in different types of care, such as males having a tendency to provide less direct care than females under biparental conditions(Smiseth & Moore 2004). The proportion of time spent caring by the parent also had a negative effect on the coefficient of variation of larval mass. Increasing levels of parental care may result in a more even distribution of food between larvae, such as by parents selectively feeding smaller larvae or those that beg most vigorously, resulting in less variation in larval mass. Precisely how parental care affects variation in larval mass in burying beetles would be an informative area of further enquiry. The coefficient of variation of larval mass was greater on smaller carcasses, which may be due to there being increased competition between larvae when there is lower food availability, resulting in greater variation in larval mass.

These results detailing costs to high mating rate in burying beetles are consistent with studies on other species. For instance, increased mating rate has been found to have a negative effect on the probability of successfully reproducing and offspring mass for the spider *Stegodyphus lineatus* (Maklakov et al. 2005).

SEX DIFFERENCES IN THE COSTS OF A HIGH MATING RATE

These results provide evidence for the existence of sex differences in the cost of a high mating rate, as predicted by Head et al. 2014. There was a significant effect of an interaction between selection regime and the sex of the parent caring on the proportion of time spent providing direct care. On average, low mating rate line males spent less time providing direct care than females, while the reverse was true for the high mating rate line (Table 1).This result fits with the prediction that the cost of a high mating rate should be relatively larger for females than males.

There was also a significant interaction between selection regime and the sex of the parent providing care on mean larval mass. On average, for the low mating rate line mean larval mass was greater for males than females, while the reverse was true for the high mating rate line. This result indicates that mean larval mass for larvae raised by females is relatively greater compared to those raised by males in the high mating rate line than in the low mating rate line. If, as predicted, costs to high mating rate result in a decrease in larvae survival, and there is a trade-off between larval number and mass, one would expect mean larval mass to be relatively higher for the sex that experiences the greatest costs of a high mating rate. This result is therefore consistent with females experiencing greater costs to high mating rate than males.

In the alternative models using mating rate instead of selection regime, there was no significant effect of an interaction between mating rate and the sex of the parent providing care on either direct care or mean larval mass. The sex of the parent providing care as a main effect did not have a significant effect on either direct care or mean larval mass in the alternative model, and mating rate as a main effect did not have a significant effect on direct care. This raises the possibility that the significant interaction in the original models may be due to long term selection on mating rate and not due to the actual costs of mating rate *per se*. This does not necessarily mean that a high mating rate is not costly. It may be that the costs of a high mating rate last throughout an individual's lifetime (rather than, for example, a short term constraint in the number of larvae that can be produced in one breeding round). The parental care behaviour of the high mating rate line may therefore have evolved to adapt to these costs of

a high mating rate, but the costs of each mating do not constrain an individual's behaviour over one breeding round.

EFFECT OF CARCASS SIZE ON THE COSTS OF A HIGH MATING RATE

There was evidence for carcass size affecting the costs of a high mating rate. There was a significant effect of an interaction between carcass size and selection regime on the proportion of larvae surviving to dispersal. For both selection lines, the average proportion of larvae surviving to dispersal was lower on small carcasses, as expected from previous studies (Smiseth et al. 2014; Hopwood et al. 2014) (Table 1). However, on average, the decrease in survival on small compared to large carcasses for the high mating rate line is smaller than those for the low mating rate line. This suggests that the costs to high mating rate are greater on large carcasses.

There was a significant effect of an interaction between selection regime and carcass size on the proportion of time the parent spent providing direct care. On average, the low mating rate line showed a decrease in mean proportion of time providing direct care on small compared to large carcasses, while the reverse was true for the high mating rate line (Table 1). This indicates that the high mating rate lines individuals spent more time providing direct care on small carcasses than expected given their behaviour on large carcasses. This therefore suggests that the costs to high mating rate are relatively greater on large carcasses.

Previous studies on burying beetles have revealed how carcass size can affect the costs of parental behaviour. For instance, the costs of increased brood size on larval mass are greater on smaller carcasses (Smiseth et al. 2014). It is therefore surprising to find in this study that the costs to mating are relatively greater on larger carcass. However, studies on other species have also found that the costs to a high mating rate can be greater with greater resource availability. Only at high nutrition levels do *Drosophila melanogaster* females show a significant cost of mating on egg production (Chapman & Partridge 1996). These results therefore support the prediction that high mating rate line individuals are unable to take full advantage of the extra resources of a large carcass.

Neither the interaction between mating rate and carcass size or mating rate as a main effect were significant in the alternative model for either proportion of larvae surviving to dispersal or the proportion of time providing direct care. In addition, carcass size as a main effect did not have a significant effect on the proportion of time the parent spent providing direct care. Again, this suggests that the significant interaction between selection line and carcass size in the original models may be due to long term selection on mating rate and genetic divergence between lines, rather than due to costs of mating to an individual *per se*.

CONCLUSION

The results from this study provide evidence for costs of mating in burying beetles. A high mating rate had a negative effect on the number of larvae initially produced and on the number of larvae surviving to dispersal. Individuals from the high mating rate lines had a tendency to spend more time providing care, suggesting there may be a trade-off between investment in egg production and parental care for females. High mating rate had a positive effect on larval mass but a negative effect on larval survival, matching previous studies which have shown a trade-off between larval mass and number (Smiseth et al. 2014). There was evidence of costs of high mating rate being greater for females than males on the proportion of time spent providing direct care and mean larval mass. There was also evidence for costs of high mating rate being greater on large carcasses for the proportion of time providing direct care and the proportion of larvae surviving to dispersal.

These results fit within a larger picture of understanding the relationship between the evolution of mating and parental care traits. Differences between sexes in the cost of mating, as identified in this study, can have consequences for the expression and evolution of parental care and for offspring fitness. This study also identified how the costs of a high mating rate can be dependent on resource availability, demonstrating the importance of measuring costs to mating and parental care under different environmental conditions. Future studies on burying beetles could focus on how parental care can reduce variation in offspring mass, as this could have important consequences for

fitness. This study focused on the costs and consequences of a high mating over one breeding round. How increased mating rate affects lifespan and lifetime reproductive success could be the focus of future enquiry.

2.5 REFERENCES

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2.6 APPENDICIES

ADDITIONAL RESULTS USING MATING RATE INSTEAD OF SELECTION REGIME

EFFECT OF MATING RATE, FEMALE SIZE AND CARCASS SIZE ON THE NUMBER OF LARVAE PRODUCED

The interaction between carcass size and mating rate was not significant, nor was the interaction between carcass size and female size, on the number of larvae initially produced (both $P > 0.16$). The size of the female had a significant

positive effect on the number of larvae initially produced ($F_{1,250}$ = 4.08, P = 0.044), whereas an increased mating rate had a significant negative effect ($F_{1,250}$ = 10.15, P = 0.002).The size of the carcass did not have a significant effect on the number of larvae initially produced ($F_{1,249}$ = 1.4, P = 0.238). (Table 2)

EFFECT OF MATING RATE, SIZE AND SEX OF PARENT PROVIDING CARE AND CARCASS SIZE ON PARENTAL CARE

The interaction between mating rate and the sex of the parent providing care was not significant, nor was the interaction between mating rate and carcass size, on the proportion of time a parent spent providing direct care (both P > 0.37). The effect of carcass size was marginally non-significant, with the proportion of time spent providing direct care being lower on smaller carcasses ($F_{1,250}$ = 2.82, P = 0.094). The sex of the parent providing care was also marginally non-significant, with males spending less time proving direct care than females ($F_{1,251}$ = 2.63, P = 0.106). There was no significant effect of the size of the parent providing care ($F_{1,248}$ = 0.88, P = 0.349), or of mating rate ($F_{1,249}$ = 1.7, P = 0.194), on the proportion of time spent providing direct care. (Table 2)

The interaction between mating rate and the sex of the parent providing care was not significant, nor was the interaction between mating rate and carcass size, on the proportion of time a parent spent providing indirect care (both P > 0.5). The size of the parent had a significant negative effect on the proportion of time they spent providing indirect care ($F_{1,250}$ = 4.04, P = 0.046). Males spent a significantly lower proportion of time providing indirect care than females ($F_{1,250}$ = 26.71, P < 0.0001). There was no significant effect of mating rate ($F_{1,248}$ = 0.07, P = 0.791), or carcass size ($F_{1,249}$ = 0.64, P = 0.423), on the proportion of time spent providing indirect care. (Table 2)

The interaction between mating rate and the sex of the parent providing care was not significant, nor was the interaction between mating rate and carcass size, on the total proportion of time that a parent spent providing care (direct plus indirect) (both P > 0.2). The size of the parent providing care had a significant negative effect on the total proportion of time spent providing care ($F_{1,250}$ = 5.43, P = 0.021). Males spent a significantly lower proportion of time

providing care than females ($F_{1,250}$ = 30.97, P < 0.0001). There was no significant effect of mating rate ($F_{1,248}$ = 0.95, P = 0.33), or carcass size ($F_{1,249}$ = 2.21, P = 0.138), on the proportion of time spent caring. (Table 2)

EFFECT OF MATING RATE, SIZE AND SEX OF PARENT PROVIDING CARE, CARCASS SIZE AND PARENTAL CARE ON LARVAL PERFORMANCE

The interaction between mating rate and the sex of the parent providing care was not significant, nor was the interaction between mating rate and carcass size, on the proportion of larvae surviving to dispersal (both P > 0.41). There was a significant effect of carcass size, with a lower proportion of larvae surviving on smaller carcasses ($F_{1,249}$ = 15.17, P = 0.0001). There was also a significant effect of the sex of the parent providing care, with males having a tendency to have fewer larvae surviving to dispersal ($F_{1,249}$ = 6.96, P = 0.009). The proportion of time spent caring by the parent had a significant positive effect on the proportion of larvae surviving ($F_{1,249}$ = 8.7, P = 0.003). The effect of mating rate was marginally non-significant, with those with higher mating rates having a tendency towards having fewer larvae survive to dispersal ($F_{1,248}$ = 3.42, P = 0.066). There was no significant effect of the size of the parent providing care on the number of larvae surviving to dispersal ($F_{1,247}$ = 1.02, P = 0.314). (Table 2)

The interaction between the sex of the parent providing care and mating rate was not significant, nor was the interaction between carcass size and mating rate, on the time taken for larvae to disperse from the carcass (both P > 0.66). The effect of the sex of the parent providing care was only marginally non-significant ($F_{1,249}$ = 3.24, P = 0.073), with larvae raised by males having a tendency to take longer to disperse. The proportion of time the parent spent caring (direct plus indirect) also had a marginally non-significant effect, with dispersal time increasing with proportion of time caring ($F_{1,250}$ = 3.15, P = 0.077). The effect of mating rate was marginally non-significant, with the offspring of individuals with a higher mating rate having a tendency to take less time to disperse ($F_{1,251}$ = 3.1, P = 0.08). Neither carcass size ($F_{1,247}$ = 0.97, P = 0.326), or the size of the parent providing care ($F_{1,248}$ = 1.45 , P = 0.23), had a significant effect on the time taken for larvae to disperse. (Table 2)

The interaction between carcass size and mating rate had a marginally non-significant effect on mean larval mass ($F_{1,242}$ = 3.83, P = 0.051). Larval mass of individuals with a higher mating rate was relatively higher compared to those with a lower mating rate on smaller carcasses than on larger carcasses. The interaction between the sex of the parent providing care and mating rate was not significant (P > 0.25). Mean larval mass was significantly decreased on small carcasses compared to large carcasses ($F_{1,245}$ = 85.48, P < 0.0001). Mating rate had a significant positive effect on mean larval mass ($F_{1,245}$ = 9.31, P = 0.003), as did proportion of time spent caring by the parent ($F_{1,245}$ = 40.99, P < 0.0001). The size of the parent providing care had a marginally non-significant positive effect on mean larval mass ($F_{1,243}$ = 3.47, P = 0.064). The sex of the parent providing care had a marginally non-significant effect, with mean larvae mass increased under male compared to female care ($F_{1,244}$ = 3.54, P = 0.061). (Table 2)

The interaction between the sex of the parent providing care and mating rate was not significant, nor was the interaction between carcass size and mating rate, on the coefficient of variation of larval mass (both P > 0.14). There was a significant effect of carcass size, with the coefficient of variation of larval mass being lower on small carcasses ($F_{1,244}$ = 29.03, P < 0.0001). Increasing size of the parent providing care had a significant negative effect on the coefficient of variation of larval mass ($F_{1,244}$ = 5.9, P = 0.016), as did the proportion of time the parent spent caring ($F_{1,244}$ = 12.36, P = 0.001). There was no significant effect of mating rate ($F_{1,242}$ = 0.05, P = 0.816) or the sex of the parent providing care ($F_{1,243}$ = 0.27, P = 0.601), on the coefficient of variation of larval mass. (Table 2)

Response Variable	Terms in Minimal Model	Model Coefficients (+/- SE)	t value	df	Pr(>|t|)
Number of Larvae Initially Produced	Intercept	6.48 (9.32)	0.7	250	0.487
	Size of Female	3.93 (1.94)	2.02	250	0.044
	Mating Rate	-0.96 (0.3)	-3.19	250	0.002
Proportion of Time Providing Direct Care	Intercept	-1.77 (0.06)	-29.46	252	< 0.0001
Proportion of Time Providing Indirect Care	Intercept	2.08 (0.78)	2.67	250	0.008
	Size of Parent Providing Care	-0.32 (0.16)	-2	250	0.047
	PC.Sex (Male)	-0.57 (0.11)	-5.14	250	< 0.0001
Proportion of Time Providing Care (Total)	Intercept	1.27 (0.21)	6.04	250	< 0.0001
	Size of Parent Providing Care	-0.1 (0.04)	-2.33	250	0.021
	PC.Sex (Male)	-0.17 (0.03)	-5.57	250	< 0.0001
Proportion of Larvae Surviving to Dispersal	Intercept	1.57 (0.27)	5.83	249	< 0.0001
	Carcass Size (Small)	-0.57 (0.15)	-3.85	249	0.0001
	PC.Sex (Male)	-0.42 (0.16)	-2.62	249	0.009
	Proportion of Time Caring	0.84 (0.28)	2.98	249	0.003
Time taken for Larvae to Disperse	Intercept	101.38 (0.98)	103.4	252	< 0.0001
Mean Larval Mass	Intercept	0.13 (0.005)	29.31	245	< 0.0001
	Carcass Size (Small)	-0.02 (0.003)	-9.25	245	< 0.0001
	Mating Rate	0.002 (0.0006)	3.05	245	0.003
	Proportion of Time Caring	0.03 (0.01)	6.4	245	< 0.0001
Coefficient of Variation Larval Mass	Intercept	0.29(0.05)	5.72	244	< 0.0001
	Carcass Size (Small)	0.04 (0.01)	5.39	244	< 0.0001
	Size of Parent Providing Care	-0.02 (0.01)	-2.43	244	0.016
	Proportion of Time Caring	-0.05 (0.01)	-3.52	244	<0.001

Table 2. *Analysis of number of larvae initially produced, proportion of time spent providing parental care (direct, indirect or both) and larval performance traits. Numbers in brackets are the standard errors of the model coefficients. PC.Sex is the sex of the parent providing care. Proportion of Time Caring is the proportion of time the parent spent providing care in total (direct plus indirect care).* Proportion time providing direct care, proportion of time providing indirect care and proportion of larvae surviving to dispersal were analysed with a quasibinomial error structure.

CHAPTER 3: PLASTICITY IN FEMALE PARENTAL CARE IN RESPONSE TO VARIATION IN THE COMPETITIVE ENVIRONMENT

ABSTRACT

Parental care can be plastic in relation to both the social and non-social environment. Plasticity in response to changes in the social environment, the presence and behaviour of members of the same species, is of particular importance, as the social environment can evolve. This means that traits expressed by interacting individuals, such as male and female partners or parents and offspring, are not independent and can coevolve. The aim of the current study was to investigate whether female burying beetle, *Nicrophorus vespilloides*, parental care duration and quality is plastic in response to the presence or absence of a rival female prior to breeding. Although the presence of same sex rivals is an important element of the social environment for burying beetles for both sexes, the consequences of female-female competition has received relatively little attention in this species compared to male-male competition. Larger burying beetles are better able as adults to win contests with other members of their sex to gain access to a carcass on which to breed. Adult size is highly dependent on larval mass, which is heavily influenced by parental care received. I investigated whether females can adaptively match the size of their offspring to the social environment that they are likely to experience. Focal females used in this study came from lines selected for high or low mating rate. Consistent with adaptive matching of offspring phenotype, females that experienced a rival produced heavier larvae, that are expected to be more competitive as adults in gaining access to a carcass. The increase in average larval mass for females that experienced a rival was probably in part due to their significantly smaller brood size, leading to a reduction in competition between larvae for food and parental care. Surprisingly, there was no evidence for genetic variability in this plasticity in parental care (i.e., no GXE), and the effect of the presence of a rival on a female's parental behaviour was independent of her size relative to the rival.

3.1 INTRODUCTION

A trait is described as being phenotypically plastic if the same genotype produces different phenotypes under different environmental conditions (West-Eberhard 1989). Phenotypic plasticity has a wide number of evolutionary implications, including potential effects on speciation, population survival in novel environments, the origins of new phenotypes, and for interactions between species (Miner et al. 2005; Pfennig et al. 2010). For example, phenotypic plasticity may allow many individuals of a species to colonise a novel environment by expressing a phenotype different to that of individuals in the old environment. This can facilitate diversification and speciation by impacting selection on related traits (e.g. a change in feeding behaviour influencing selection on morphology), and by promoting reproductive isolation (e.g. morphs that feed on different food types may be active at different times of day and in different places, resulting in isolation between them) (Pfennig et al. 2010). Over time, the induced phenotype in the population living in the novel environment can become genetically fixed, expressed regardless of the environment experienced, and alleles regulating the expression of the alternative phenotype may be lost due to genetic drift (Pfennig et al. 2010). If the extent of plasticity of a trait is to respond to selection and evolve it needs to be heritable, and this requires different genotypes to differ in their response to variation in the environment (a 'gene by environment interaction (GxE))'(Nussey et al. 2007; Dingemanse & Wolf 2013). Knowledge of whether there is genetic variability in the extent of plasticity of different traits is therefore essential for understanding phenotypic plasticity's wide ranging implications for evolution.

Parental care is an example of a trait that can be highly plastic in relation to various aspects of the social and non-social environment (Royle et al. 2014). For instance, a parent may spend less time provisioning offspring when predators are more abundant (non-social environment), so as to not reveal their location or to spend more time guarding. A study of 10 bird species from Arizona and Argentina found that parents from all species reduced their provisioning of offspring in response to recordings of vocalisations of a predator. Those species that experience a higher natural predation risk were the most responsive to the predator stimulus (Ghalambor et al. 2013). An individual's

parental care is often influenced by the behaviour of their partner and offspring (the social environment). For example, male burying beetles, *Nicrophorus vespilloides*, spend more time provisioning larvae in the absence of their partner (Smiseth et al. 2005). Parental care is a particularly important trait to study as it is found in numerous different species, ranging from mammals to invertebrates. It can also take a wide variety of different forms, including providing eggs with yolk, guarding nests and provisioning offspring with food (Smiseth et al. 2012; Royle et al. 2014), and can have a large impact on offspring phenotype and fitness (Alonso-Alvarez & Velando 2012). To date, most research has focused on plasticity in parental care in relation to the non-social environment. However, an important aspect of the social environment is that it can evolve, as the phenotype of other individuals in the population is influenced by their own genes, which can also respond to selection (Moore et al. 1997).This can lead, for instance, to the coevolution of parent and offspring traits (Royle et al. 2014). Studies of plasticity in parental care in relation to the social environment are therefore crucial to our understanding of observed patterns of parental care, as well as being informative regarding the evolution of phenotypically plastic traits more generally.

Phenotypic plasticity can be maladaptive, neutral or adaptive (West-Eberhard 1989; Nussey et al. 2007). For adaptive plasticity to evolve there needs to be a reliable cue of future environmental conditions. Parents often have access to information regarding the environmental conditions that their offspring will experience (Uller et al. 2013). Therefore it may be possible for parents to alter their parental care so as to adaptively match their offspring's phenotype to the selective conditions they will experience. Anticipatory parental effects occur when parents transmit information regarding the environment to offspring, leading to an alteration of offspring phenotype to increase their fitness in the same (or otherwise predicted) conditions. This leads to offspring having highest fitness in the environment that their parent's environment predicted. There is evidence from European earwigs, *Forficula auricularia*, for anticipatory parental effects in relation to the nutritional environment (Raveh et al. 2016). The offspring of female earwigs were cross-fostered to females experiencing either the same or different food availability (high or low) as their mother. In the low food foster environment, late juvenile survival was significantly higher for the

offspring of mothers who experienced low food availability than those that experienced high food availability. The reverse was true in the high food foster environment, but this result was not significant when clutch size was accounted for, indicating that adjustments to clutch size by the female can increase offspring survival when parent and offspring environment match.

Adaptive phenotypic plasticity is not the only evolutionary response to environmental variability. Another strategy is 'bet-hedging', and involves traits which maximise geometric-mean fitness (long term fitness over multiple generations) by having low variance in fitness across generations, but which do not have the highest fitness over a single generation (Simons 2011). Bet-hedging is likely to evolve when environmental conditions change dramatically over short periods of time (preventing adaptive tracking - a trait evolving gradually over generations to follow the optimum), and are unpredictable (preventing the evolution of adaptive phenotypic plasticity)(Simons 2011). There are two forms of bet-hedging; conservative and diversifying. In conservative bet-hedging a single 'safe' phenotype is produced, which has a low risk of complete reproductive failure and hence high fitness over multiple generations (Simons 2011). For example, large individuals may have an advantage in years of good food availability but have an increased risk of mortality in poor years; medium sized individuals may therefore have higher long term fitness and hence be selected for. In contrast, diversifying bet-hedging reduces the risk of complete failure by producing a range of phenotypes (e.g. offspring of a range of sizes). An example of a diversification bet-hedging trait which has been studied in detail is the timing of seed germination in the perennial herb *Lobelia inflata*. In one experiment, newly germinated seedlings were transferred to the field over five seasons, and their survival recorded to generate a fitness landscape. Fluctuations in selection were sufficient to explain variance in the timing of germination, providing evidence for adaptive bet-hedging (Simons 2009).

Burying beetles, *Nicrophorus vespilloides,* display varied parental care which is plastic in response to a number of different environments. This makes them an ideal species in which to investigate plasticity in parental care in relation to the social environment. Burying beetles remove the fur from a small vertebrate carcass, such as a bird or mouse, before rolling it into a ball and burying it

(Eggert & Müller 1997; Scott 1998; Royle et al. 2013). Females then lay eggs in the surrounding soil, and once hatched the larvae crawl into a crater prepared by the parents on the carcass. Either parent can provide care alone (uniparental care) or together (biparental care), which can range from indirect care (such as preparing carcass, guarding from competitors) to direct care (direct feeding of larvae) (Royle et al. 2013). Parental care has a positive effect on larval growth and survival (Eggert et al. 1998). This is important, as body size has a large influence on burying beetles' competitive ability as an adult to gain access to a carcass, with the larger individual nearly always gaining the resource (Otronen 1988).

Much research has been carried out on plasticity in burying beetle behaviour in relation to the non-social environment. For example, female burying beetles lay a greater number of eggs on larger carcasses (Müller et al. 1990). Both male and female burying beetles can experience fierce competition with members of their sex over control of a carcass, making it an important element of their social environment. A recent study investigating plasticity in male burying beetles found that they spend significantly less time signalling for females in the presence of a competitor (Carter et al. 2015). The focal beetles used in this study came from lines selected for either high or low mating rate. High mating rate is a paternity assurance trait, expected to evolve in order to increase probability of paternity of offspring when males experience high competition with other males over access to females (Müller & Eggert 1989; Head et al. 2014). There was a GxE for male activity on the carcass, with males from the high mating rate lines being more sensitive to the social environment than those from the low mating rate lines.

Selection due to competition has been shown to have wide ranging evolutionary implications. The same burying beetle selection lines for high and low mating rate were also used in another study to investigate the co-evolution of parental care and mating rate (Head et al. 2014). It was found that the selection regime did not result in a change in male parental care, but that female parental care had co-evolved in response to selection on males, with high mating rate line females providing lower levels of care. This was suggested to be due to the cost of a higher mating rate on females.

The consequences of male-male competition on male behaviour, male and female parental care and offspring performance in burying beetles has been researched in depth (Head et al. 2014; Carter et al. 2015). However, despite its importance for female reproductive success, relatively little is known about the consequences of female-female competition in burying beetles on parental care and offspring performance. As females tend to care for offspring for longer than males, and are more likely to be the sole carer under uniparental care (Bartlett 1988; Eggert & Müller 1997; Scott 1998), female competition may have important implications for offspring phenotype and fitness. A female's parental care could be impacted due to the stress of fighting, or they may be able to use information on the social environment to increase their offspring's fitness through anticipatory parental effects. A recent study on female burying beetle competition found that whether a female won or lost a contest for a carcass did not affect her later parental care or larvae performance (Pilakouta et al. 2016). However, the experience of competing with a rival prior to reproduction did significantly affect parental care, with females that experienced a rival providing more direct care and producing larger broods (Pilakouta et al. 2016). This was suggested to be due to females conserving resources for future reproduction when population density is low and the chances of breeding again are high. However, Pilakouta et al. (2016) did not investigate whether there was genetic variability in this plasticity in female parental care in response to the presence or absence of a rival, so it is unclear whether there would be a strong evolutionary response to selection on this plasticity. In addition, the study did not investigate the effect of the social environment experienced by the female on within-brood variation in larval mass, a potential bet-hedging trait.

The aim of the current study was to investigate whether there is plasticity in female burying beetle parental care in response to variation in the competitive environment experienced, or whether they use a bet-hedging strategy. This will enable a better understanding of the impact of female competition in burying beetles, as well as of plasticity in relation to the social environment more generally. Specifically, is female burying beetle parental care plastic in response to the presence or absence of rival females prior to breeding? Is there genetic variation in this plasticity (a GxE)? As discussed above, optimum body size is likely dependent upon the social environment. Large individuals have an

advantage in competition over carcasses, but smaller males appear to have an advantage when carcasses are rare and competition less intense, as they are better able to locate a carcass and not attract rivals (Hopwood, Moore, et al. 2016). Through altering her parental care, it may be possible for a female burying beetle to adaptively match the size of her offspring to the competition environment they will likely experience. For example, the presence of a rival female may indicate a high population density and therefore high competition for carcasses. This could lead to the focal female increasing larvae size by changing the type of parental care she provides (e.g. direct vs indirect), providing care of higher quality or of greater duration/frequency, or by increasing the resources available to each offspring by reducing brood size. These heavier larvae would then be better able as adults to compete for access to a carcass. By contrast, in the absence of competitors, a female may alter her parental care so as to produce small offspring, making them better able as adults to locate and retain carcasses (Hopwood, Moore, et al. 2016). In these circumstances, as producing larger larvae is less advantageous, females may display lower parental effort (e.g. lower quality, less frequent or shorter duration of care) so as to conserve resources for future reproduction, as suggested by the results of Pilakouta et al. (2016).

It may be that, instead of being plastic in their parental care, females respond to variation in the social environment by using a diversification bet-hedging strategy, producing larvae of a range of sizes regardless of the competition environment they experience. I predict a diversification bet-hedging strategy rather than a conservative strategy, as burying beetle larvae within a brood can differ considerably in mass, and this can have a significant effect on their size as an adult and fitness (Bartlett & Ashworth 1988; Otronen 1988). Using a bet-hedging strategy seems plausible, as the competition environment that a parent experiences may be different to that of their offspring, and is therefore unlikely to be a highly reliable cue to which phenotype will be most adaptive in the offspring. However, the extent of bet-hedging may be influenced by the social environment that a female experiences. I predict that females who experience competition may show greater variability in larval mass for two reasons. Firstly, it is possible that, if a female doesn't experience a rival, it may signal a very low population density and a low probability that her offspring will experience

competition, meaning that there is only limited need for bet-hedging. In contrast, if the female experiences a rival, it could signal that population density is anywhere between moderate to very high. In addition, crashes in population density, such as due to poor weather conditions, may be much quicker than population growth under good conditions. Therefore, if a female experiences a rival, there may be more uncertainty as to the population density and extent of competition her adult offspring may experience, leading to greater bet-hedging and hence greater variation in larval mass. Secondly, It could also be that, in the presence of a rival, females may not be able both adaptively increase average larval mass and ensure equal distribution of resources between larvae. It may only be possible for a female to produce a few very large larvae by sacrificing the size of other larvae in the brood.

Competition may also result in a female burying beetle experiencing stress, such as physical injury due to fighting. This could lead to the female being in a worse physical condition, and unable to produce as many eggs or to provide as much care to larvae. Therefore, contrary to the adaptive matching hypothesis above, females experiencing competition may alternatively show a reduction in average larval mass and/or number of larvae surviving to dispersal. However, the results from Pilakouta et al. (2016) suggest that this is unlikely to be the case as, in their study, female burying beetles which experienced a rival produced larger broods than those that didn't.

There is also the possibility that females may use the size of the rival female to gauge their own size in relation to that of the rest of the population. If there is adaptive phenotypic plasticity, and females match their offspring's phenotype to the competition environment, one would expect this effect to be magnified in the presence of rival females relatively larger in size to the focal female. There is also the possibility that pairs of beetles that differ greatly in size may interact differently with each other than those of similar size. For example, a focal female may be able to quickly dispel a much smaller rival from the carcass, resulting in the encounter having little effect on her later parental care. However, as contest outcome, which is highly dependent on a individual's size relative to their competitor, was found to have no significant effect on female

parental care in the Pilakouta et al. (2016) study, it would appear unlikely for the relative size of the rival to have a significant effect in this present study.

In order to test these predictions I used the following experimental design. Prior to breeding, female beetles were placed in a breeding box for 48 hours, either alone or with a female competitor. Males were removed prior to larvae hatching to produce uniparental female care. The duration of parental care of each focal female was measured, along with larval performance and within brood variation in larval mass. To test for genetic variability in plasticity (a GxE), focal females came from lines selected for either high or low mating rate (Head et al. 2014). The competitive interactions between focal and rival female during the 48 hours together were not quantified during the experiment. However, although the full implications of female-female burying beetle competition are not fully understood, it is well established that females do interact, with the larger individual usually gaining control of the carcass (Eggert & Müller 1997; Pilakouta et al. 2016). This study primarily differs from that of Pilakouta et al. (2016) in a) using genetically diverged selection lines in order to test for a GxE in plasticity in response to the social environment, and b) measuring the effect of the social environment experienced by the female on within-brood variation in larval mass, a potential bet-hedging trait.

My predictions are that females in the rival treatment group will produce broods with greater average larval mass, potentially by providing more parental care. If there is a GxE (i.e. the selection lines differ in their plasticity), I predict that females from the high mating rate lines will show the greatest increase in parental care in response to the presence of a rival female. This is because a) males from the high mating rate lines are more plastic in their signalling behaviour (to attract a female) than those from the low lines in response to the social environment (Carter et al. 2015) and b) selection for high mating rate effectively selects for dominant individuals to be more responsive to the competition environment (Carter et al. 2015). Following the results of Pilakouta et al. (2016), I predict that the relative size of the focal female to the rival will not have a significant effect on her parental care. If the social environment experienced does impact within brood variation in larval mass, I predict it will be more variable for females that experienced a rival.

3.2 METHODS

ORIGIN AND MAINTENANCE OF BEETLE SELECTION LINES

This study was carried out on generation F40 of burying beetle (*Nicrophorus vespilloides*) lines selected for either high (H) or low (L) mating rate. There were two replicate high and low lines (H1, H2, L1, L2). Selection on mating rate was carried out for 24 generations in the lab by conducting mating trials and choosing the 30% of pairs with the highest or lowest mating rate to breed. From this point the lines were maintained in the lab with no selection. To breed, non-sibling male-female pairs were placed in transparent plastic breeding boxes (17 x 12 x 6 cm), filled with 2cm of moist compost and a mouse carcass. Once larvae had dispersed from the carcass they were placed in individual plastic pots (7.0 x 7.0 x 4.0 cm or 10.8 x 8.2 x 5.0 cm). Post-eclosion, beetles were fed two mealworms (*Tenebrio moliter*) twice weekly. Beetles from generation F40 of two control lines, which had undergone no selection, (C1, C2) were also used in the experiment. All lines were established from beetles collected in July 2010 in Devichoys Wood, Cornwall, UK (N 50º11'47'' E-5º7'23''). For more information on the origin of the selection lines see (Head et al. 2014; Carter et al. 2015; Hopwood, Head, et al. 2016).

EXPERIMENTAL DESIGN

The experiment started when beetles were between 10 and 19 days post eclosion, which is after the point maturity is reached. Focal females came from one of the high or low mating rate lines, and were marked prior to the experiment by gently sanding a small patch of the posterior left dorsal side of their elytra with a drill and applying a small amount of tip-pex. Rival females came from one of the control lines and had their elytra sanded in the same position as the focal females, but were not marked with tip-pex. All females had their pronotum width measured twice to 0.1mm using callipers and a mean value was calculated. All females were handled for the same amount of time.

Focal females were placed in a transparent plastic breeding box (17 x 12 x 6 cm), which had been filled with 2cm of moist compost (Verve Multipurpose Compost, 42% peat free) and a moist mouse carcass. Mouse carcasses were defrosted for 24 hours before use; they were measured to 0.01g using a Ohaus

ScoutPro Balance, and had a mass between 15.0-20.1g (mean 18.3g +/- 0.077 SE). There were two treatment groups; focal females were either left alone in the breeding box or with a rival female from a control line for 48 hours. At this point rival females were removed, where present, and a male from the same line as the focal female was added to all breeding boxes. The focal female and male were non-siblings, and females from the same family (sisters) were not paired with males who came from the same family as each other (brothers), in order to prevent inbreeding and ensure independence between pairs. 56h later each breeding box was checked for the presence of eggs. From this point onwards boxes were checked twice daily at 9h and 17h, and males were removed once eggs were visible, to ensure they were not present when larvae hatched (Head et al. 2014). At each observation the location of the female either on or away from the carcass at this single time point, and the presence/absence of larvae, was recorded. Observations continued until larvae had dispersed from the carcass, which was defined as the point at which two larvae were present in the soil away from the carcass (Head et al. 2014). The duration of parental care was defined as being from the point at which larvae appeared to the point at which the female had been observed away from the carcass for two consecutive observations (Head et al. 2014). The proportion of time that each female cared for offspring was calculated as the duration of parental care divided by time taken from larvae first appearing on the carcass to their dispersal.

During the experiment beetles were kept in an incubator at 21°C, with a 16:8 light:dark cycle. The experiment started for each of the replicate lines on different days, with H1 the first, followed two days later by H2, a further 2 days later by L1, and a further two days later by L2. The initial sample size for each line across both treatments was as follows; 56 H1, 54 H2, 50 L1 and 49 L2.

At dispersal, soil was removed from larvae by placing them on a paper towel, spraying them gently with water, and drying with a clean paper towel on top. The number of surviving larvae was recorded and the total brood mass was measured to 0.001g using a Ohaus Explorer digital balance in order to calculate average larval mass. From each family the mass of 10 individual larvae was also measured to 0.001g. These larvae were randomly selected by placing the

whole brood onto a transparent piece of 17 x 6cm flat plastic, and choosing those that landed above one of 20 randomly arranged 1cm^2 squares of tape on the underside. In broods with fewer than 10 larvae all were measured (37 out of the 125 successful broods). The coefficient of variation of larval mass for each brood was calculated as the standard deviation in larval mass divided by the average mass of the total number of individually measured larvae.

MATING TRIALS

Mating trials were conducted on generation F39, the parent generation of the beetles used in the main study. This was to ensure that the mating rate of the high and low lines had not converged in the absence of continued selection after generation 24. Mating trials were conducted for 35 pairs of beetles for C1 and 20 pairs for each of the other 5 lines. Each mating trial involved placing a non-sibling male-female pair in an 8cm Petri dish lined with filter paper, and recording the number of copulations over 1 hour. A copulation was defined as the male inserting his aedeagus into the female (Head et al. 2014). The average mating rate for each selection line replicate were as follows, with the standard deviation in brackets; C1- 3.51 (SD 1.82), C2- 2.05 (SD 1.15), H1- 4.75 (SD 2.05), H2- 5.4 (SD 3.75), L1- 2.85 (SD 1.53), L2- 2.25 (SD 1.25).

DATA ANALYSIS

Data was analysed using R version 3.3.0 (R Core Team 2016). To investigate the effect of the social environment (presence/absence of a rival) on female parental care and offspring performance, general linear models were produced. Only families in which neither of the parents died during the experiment were included in the analysis. An exception was made for those in which larvae dispersed within 24 hours of the female's death, after parental care had already finished (1 family). The first model investigated the probability of successfully producing larvae surviving to dispersal, using a generalized linear model (GLM) with a binomial error structure and a logit link. Explanatory variables included in the model were focal female line (high or low), treatment group (rival (R) or no rival (N)) and focal female size.

For those that produced larvae surviving to dispersal, a number of response variables were analysed using linear mixed models (LMM) using the R package

lme4. These were number of larvae surviving to dispersal, average larval mass, coefficient of variation of larval mass, proportion of time caring and time taken for larvae to disperse. Proportion of time caring was analysed using a generalized linear mixed model (GLMM) with a binomial error structure and a logit link. Explanatory variables included in the models were focal female line, treatment group, focal female size, the interaction between focal female size and treatment and the interaction between focal female line and treatment. For proportion of time caring and time to dispersal, number of larvae surviving to dispersal was included as an additional explanatory variable. This was because parental care is likely to be influenced by the number of larvae in the brood and their behaviour (e.g. begging), and the time taken for larvae to disperse may be influenced by competition with siblings (Lock et al. 2004). For each model, focal female line replicate was included as a random effect, allowed to vary in intercept only. To reduce overdispersion in the binomial GLMM for proportion of time spent caring, an observation level random effect was included in the model (Harrison 2015).

General linear mixed models were also produced to analyse the effect of the relative size of the rival female on the focal female's parental care in the rival treatment group. The relative size of the focal female to the rival was calculated as (focal size-rival size)/focal size (Lee et al. 2014). These models were as above, except the explanatory variables were focal female line, focal female size, relative size of the focal female to the rival and the interaction between focal female line and relative size of the focal to the rival female. As above, for proportion of time caring and time to dispersal, number of larvae surviving to dispersal was included as an explanatory variable.

Models were fitted using the ML(maximum likelihood) method for LMMs, and the ML Laplace approximation for GLMMs. Models were simplified using backward stepwise elimination for the fixed effects, with interaction terms being tested first (Bolker et al. 2009; Crawley 2013). Only terms with a significance of $P < 0.05$ were included in the minimal model. The P-value given for variables not included in the minimal model is that at the point of their removal. The P-value given for significant terms is for their removal from the minimal model. For the LMMs, the minimal model was refitted using REML, and the significance of

the random effect tested using the package lmerTest. All LMM coefficients are from REML models. The significance of random effects of GLMMs cannot be tested using the lmerTest package.

3.3 RESULTS

EFFECT OF COMPETITION AND SELECTION REGIME ON PROPORTION OF SUCCESSFUL BREEDERS

Females from the low mating rate lines were significantly more likely to produce offspring surviving to dispersal than those from high mating rate lines ($\chi^2 = 22.4$, df = 1, $P < 0.0001$). There was no significant effect of treatment group ($\chi^2 = 0.31$, df = 1, $P = 0.577$) or focal female size ($\chi^2 = 1.05$, df = 1, $P = 0.305$) on the probability of producing offspring surviving to dispersal.

EFFECTS OF COMPETITION, FOCAL FEMALE SIZE AND SELECTION REGIME ON FEMALE PARENTAL CARE

PROPORTION OF TIME SPENT CARING AND TIME TAKEN FOR LARVAE TO DISPERSE

The interaction between focal female size and treatment was not significant, nor was the interaction between focal female line and treatment, on the proportion of time the female spent caring (both $P > 0.29$). The proportion of time spent caring significantly increased with the number of larvae in the brood (binomial GLMM, $\chi^2 = 13.01$, $df = 1$, $P = 0.0003$) (Table 1, Fig 1). There was no significant effect of focal female size ($\chi^2 = 0.1$, $df = 1$, $P = 0.748$), focal female line ($\chi^2 = 0.36$, $df = 1$, $P = 0.549$) or treatment ($\chi^2 = 1.75$, $df = 1$, $P = 0.187$), on time spent providing care.

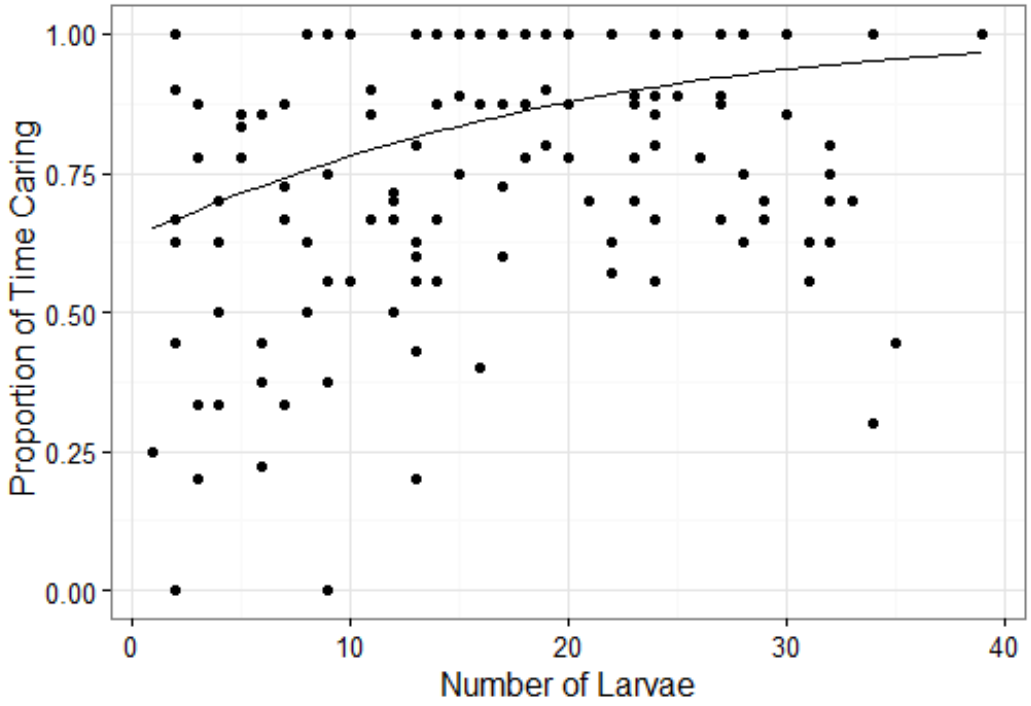

Fig 1. *The number of larvae in the brood (number surviving to dispersal) had a significant positive effect on the proportion of time the female cared for. Fitted line is that given by the minimal model.*

The interaction between focal female size and treatment was not significant, nor was the interaction between focal female line and treatment, on the time taken for larvae to disperse from the carcass (both $P > 0.15$). There was no significant effect of the number of larvae ($\chi^2 = 0.3$, $df = 1$, $P = 0.583$), focal female size ($\chi^2 = 0.42$, $df = 1$, $P = 0.518$), treatment ($\chi^2 = 1.11$, $df = 1$, $P = 0.292$) or focal female line ($\chi^2 = 1.37$, $df = 1$, $P = 0.241$), on the time taken for larvae to disperse from the carcass (Table 1).

NUMBER OF LARVAE SURVIVING TO DISPERSAL

The interaction between focal female size and treatment was not significant, nor was the interaction between focal female line and treatment, on the number of larvae dispersing from the carcass (both $P > 0.4$). Focal female size had a significant positive effect on the number of larvae ($\chi^2 = 4.41$, $df = 1$, $P = 0.036$). The effect of the competition environment was significant, with fewer larvae dispersing in the rival treatment group ($\chi^2 = 4.36$, $df = 1$, $P = 0.037$) (Table 1, Fig 2). Focal female line did not have a significant effect on the proportion of larvae surviving to dispersal ($\chi^2 = 1.38$, $df = 1$, $P = 0.24$).

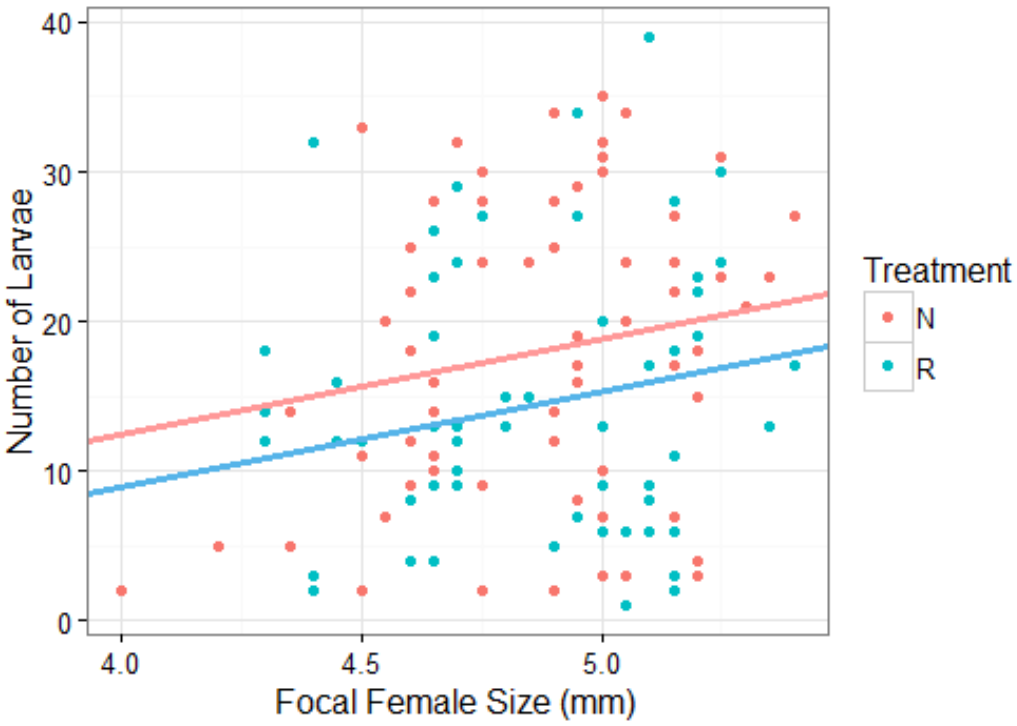

Fig 2. *Larger females and those that didn't experience a rival female produced significantly more larvae surviving to dispersal. Treatment group- No rival (N), rival (R). Fitted lines are those given by the minimal model.*

AVERAGE LARVAL MASS AND COEFFICIENT OF VARIATION OF LARVAL MASS

The interaction between focal female size and treatment was not significant, nor was the interaction between focal female line and treatment, on average larval mass (both $P > 0.45$). The competition environment had a significant effect, with females in the rival treatment group producing heavier larvae ($\chi^2 = 6.58$, $df = 1$, $P = 0.01$) (Table 1, Fig 3). The effect of focal female size was marginally non-significant, with larger females having a tendency to produce heavier larvae ($\chi^2 = 3.07$, $df = 1$, $P = 0.08$). There was no significant effect of selection regime on average larval mass ($\chi^2 = 1.31$, $df = 1$, $P = 0.253$).

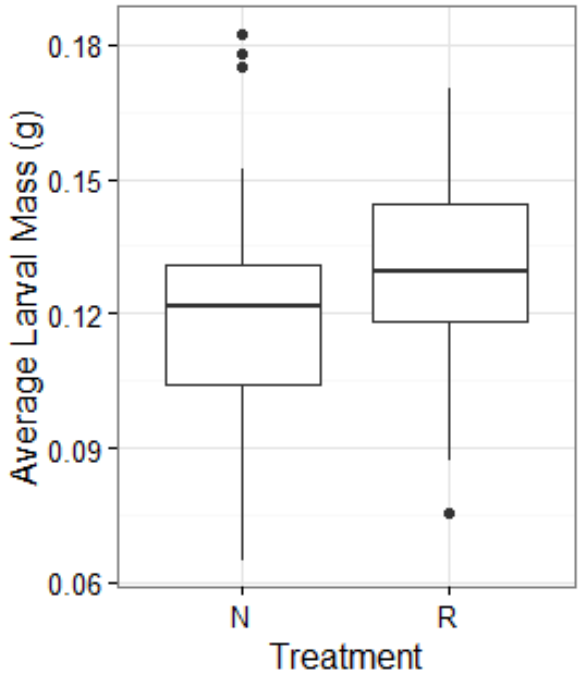

Fig 3. *Average larval mass was significantly higher for females that experienced competition (rival treatment group) than those that didn't. Treatment group- No rival (N), rival (R). Mean average larval mass(g) (+/-standard error); no rival group = 0.12(0.003), rival group = 0.13(0.003).*

The interaction between focal female size and treatment was not significant, nor was the interaction between focal female line and treatment, on the coefficient of variation of larval mass (both $P > 0.25$). There was a significant effect of focal female selection regime, with low mating rate line females showing less variability in larval mass ($\chi^2 = 4.35$, $df = 1$, $P = 0.037$) (Table 1, Fig 4). There was no significant effect of treatment ($\chi^2 = 0.09$, $df = 1$, $P = 0.763$) or focal female size ($\chi^2 = 1.05$, $df = 1$, $P = 0.305$), on the coefficient of variation of larval mass.

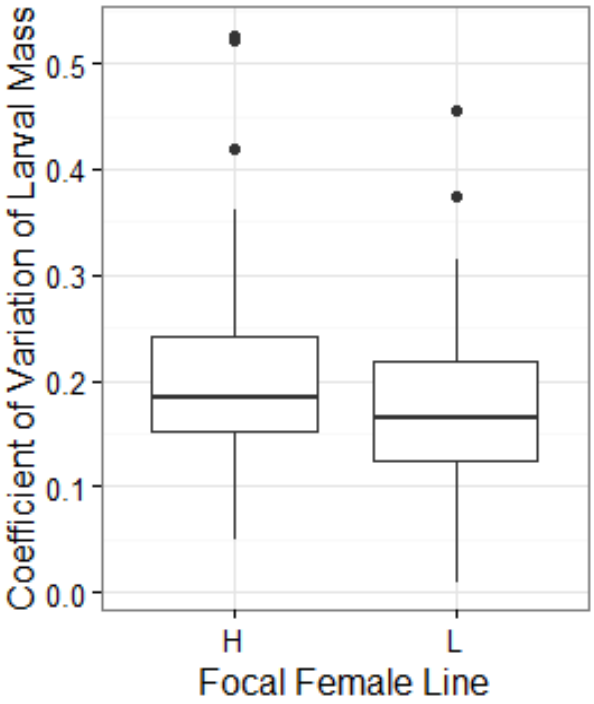

Fig 4. *The coefficient of variation of larval mass was significantly lower for low mating rate line females than for high mating rate line females. Mean coefficient of variation of larval mass (+/-standard error); high mating rate line = 0.21(0.01), low mating rate line = 0.17(0.01).*

Response Variable		**Model Coefficients** (+/- SE)		**t value/ z value/ Chi.sq**	**d.f.**	**Pr(>|t|)/ Pr(>|z|)/ p.value**
Proportion of Time Caring	Fixed Effects	Intercept	0.56 (0.55)	1.01	**	0.314
		Number of Larvae	0.07 (0.02)	3.7	**	0.0002
	Random Effects	Focal Female Line Rep		*	*	*
Time to Dispersal (h)	Fixed Effects	Intercept	100.16 (3.4)	29.46	3.08	< 0.0001
	Random Effects	Focal Female Line Rep		10	1	0.002
Number of Larvae	Fixed Effects	Intercept	-12.93 (14.38)	-0.9	118.21	0.37
		Focal Female Size	6.36 (2.95)	2.16	119.75	0.033
		Treatment (R)	-3.52 (1.69)	-2.09	120.53	0.039
	Random Effects	Focal Female Line Rep		0.22	1	0.6
Average Larval Mass (g)	Fixed Effects	Intercept	0.12 (0.004)	28.14	4.47	< 0.0001
		Treatment (R)	0.01 (0.004)	2.59	120.6	0.011
	Random Effects	Focal Female Line Rep		6.47	1	0.01
Coefficient of Variation Larval Mass	Fixed Effects	Intercept	0.21 (0.01)	16.34	122	< 0.001
		Focal Female Line (L)	-0.04 (0.02)	-2.19	122	0.03
	Random Effects	Focal Female Line Rep		0	1	1

Table 1. *Analysis of female parental care and larval performance for all those that successfully produced larvae. The fixed effects are the significant terms (P<0.05) remaining in the minimal model after backwards stepwise elimination. Numbers in brackets are the standard error of each model coefficient. All models are LMM except for Proportion of Time Caring, which is a binomial GLMM. The t values of each LMM coefficient and the z value of each GLMM coefficient are given, and the degrees of freedom were approximated using* Satterthwaite approximations*. Each model included focal female line replicate (Rep) as a random effect, the significance of which was calculated using a chi sq test.*

**The significance of the random effect could not be tested for the proportion of time caring due to the model being a binomial GLMM.*

***The degrees of freedom for the Z test of each fixed effect in the GLMM could not be calculated by the statistics package used.*

Next the rival treatment group was considered in isolation. The interaction between focal female line and the relative size of the focal female to the rival female did not have a significant effect on any of the measures of parental care or larval performance (all $P > 0.43$), with the exception of average larval mass for which the interaction was only marginally non-significant ($\chi^2 = 2.92$, $df = 1$, $P = 0.087$). Larval mass was relatively higher for low mating rate line females compared to high mating rate line females when relative focal female size was greater. The main effect of the relative size of the focal female was not significant for any of the response variables (all $P > 0.2$). None of the explanatory variables were found to have a significant effect on the number of larvae surviving to dispersal, average larval mass or time to dispersal (all $P > 0.13$). As with the previous models analysing both treatment groups, coefficient of variation of larval mass was lower for low mating rate line females ($\chi^2 = 5.13$, $df = 1$, $P = 0.023$), and proportion of time caring was greater for larger broods (binomial GLMM, $\chi^2 = 14.05$, $df = 1$, $P = 0.0002$). None of the other explanatory variables had a significant effect on the coefficient of variation of larval mass or proportion of time spent caring (all $P > 0.31$).

3.4 DISCUSSION

To date, despite its importance for the evolution of parental behaviour and the coevolution of male-female and parent-offspring traits, there has been relatively little research on phenotypic plasticity in parental care in response to variation in the social environment (Royle et al. 2014). This study furthers our understanding of plasticity in parental care in response to the social environment, as well as the consequences of female-female competition in burying beetles, which has previously received little attention. In many species, the presence of same-sex competitors is an important element of the social environment (Clutton-Brock 2007). This study presents evidence of female burying beetle parental care being plastic in response to the presence or absence of a rival female. As predicted, females that interacted with a rival female prior to breeding produced heavier larvae, suggesting they can adaptively match offspring phenotype to the competition environment they will

experience. However, there was no evidence for genetic variability in this plasticity (a GxE), suggesting that it wouldn't respond strongly to selection. Following predictions, the response to the presence of a rival female did not depend on the relative size of the focal female to the rival. The extent of within brood variation in larval mass, a potential bet-hedging trait, did not vary with the social environment experienced by the female.

This study found evidence for plasticity in female burying beetle parental care in response to the presence of a rival female. To date, little research has been carried out on the consequences of female-female competition in burying beetles. The results of this study demonstrate that this competition can have a significant effect on a female's behaviour and reproductive success even after the rival individual has been removed. Females that experienced a rival prior to the start of parental care produced significantly fewer larvae surviving to dispersal, but these larvae were of significantly greater mass on average. These heavier larvae will be better able as adults to compete with others of their sex for access to a carcass on which to breed. This is consistent with adaptive anticipatory parental effects, with females that experience the presences of a rival producing offspring most suited as adults to compete with rivals for a carcass (Uller et al. 2013; Raveh et al. 2016). For this plasticity to be adaptive, there needs to be a positive correlation between the social environment experienced by a female and her offspring, and this could be the focus of future research. Intra-sexual competition amongst burying beetles could potentially be highly variable over short time periods, such as due to the effect of changes in the weather and season on population density and carcass availability. Therefore, the correlation between the social environment experienced by a female and her offspring may be weak, and this parental effect may not be adaptive.

The increase in mean larval mass for the offspring of females that experienced a rival could be the result of the significant reduction in brood size. Increased brood size has been shown to have a negative effect on burying beetle larval mass (Monteith et al. 2012). This reduction in brood size could be a result of the female adaptively restricting egg laying or cannibalizing larvae, both of which have been observed in burying beetles (Bartlett 1987; Müller et al. 1990).

Alternatively, physical costs to interacting with a rival female may mean that the female is physically unable to produce as many eggs, or that the female is no longer able to care sufficiently for a larger brood, reducing the optimal brood size. The increase in mean larval mass may therefore be a consequence of costs to the female of interacting with a rival rather than brood size being adaptively reduced in order to increase adult offspring size. Nevertheless, these results are consistent with the existence of a previously reported trade-off between larval number and mass in burying beetles (Monteith et al. 2012; Smiseth et al. 2014).

These results contrast with those of Pilakouta et al. (2016), which found that female contest experience has no effect on average larval mass but a positive effect on brood size. Possible explanations for the differences in our results include the fact that this present study used focal females from lines selected for high or low mating rate, while Pilakouta et al. (2016) used wild type individuals. Selection on mating rate could have resulted in correlated response in parental care traits. There were also differences in the protocol between the two studies; females interacted with a rival for 3 days in the Pilakouta et al. (2016) study, and those that lost the contest for the carcass were transferred to a new one before breeding, to mimic losing a contest in the wild. In contrast, in this present study, females interacted for two days and none were transferred to a different carcass, meaning that the behaviour of females that lost the contest may be different than in the Pilakouta et al. (2016) study. In addition, the focal females used in the Pilakouta et al. (2016) study were all medium in size, whereas females of all sizes were used in this present study. It is possible that a female's response to the social environment is dependent on her size, which could account for the differences in results between these two studies. Whether there is an effect of an interaction between a female's size and the competition environment they experience on parental care could be investigated in future studies. While the results of this current study and Pilakouta et al. (2016) differ in detail, they both show that the presence of a rival female prior to breeding can shift investment by the focal female, either into producing heavier larvae or more larvae in the current breeding round.

In this current study, the social environment experienced did not have a significant effect on the duration of parental care by the female. This was surprising, as if there were anticipatory parental effects or a cost to interacting with rival females, one would expect there to be an alteration in the duration of parental care (an increase in the presence of a rival in the former in order to increase larval mass, a decrease in the latter due to energetic constraints). In addition, Pilakouta et al. (2016) found that females with contest experience spent more time providing direct care than those that did not. However, the measure of duration of parental care used in this present study was relatively simple, and did not examine the quality or frequency of care which may have differed between the treatments. Females spent significantly more time caring for broods that had more larvae surviving to dispersal; this may be due to parental care increasing larval survival and/or larger broods requiring more care. The advantage to the female of staying with the carcass and caring for larvae for longer may be greater for larger broods. For instance, early in the breeding season when there is a good possibility of the parent breeding again, the duration of female parental care of Kentish plovers, *Charadrius alexandandrinus*, is longer for larger broods, likely due to a trade-off between current and future reproduction (Szekely & Cuthill 2000). A study on burying beetles found that parents are more likely to desert small broods, freeing themselves to start the next brood (Ward et al. 2009).

There was no significant effect of an interaction between selection line and social environment experienced (rival or no rival) on any of the measures of parental care or larval performance. This suggests the absence of genetic variability in plasticity (GxE) in response to the social environment, and that the response to selection on plasticity in these traits would be limited (Nussey et al. 2007; Dingemanse & Wolf 2013). This therefore differs from the results of (Carter et al. 2015), which found a GxE for plasticity in male burying beetle activity on the carcass, with individuals from the high mating rate line being more responsive to the presence/absence of a rival male than those from the low mating rate line. It may be that optimum plasticity in female parental care in response to a rival is unrelated to mating rate, and therefore selection on mating rate has not resulted in a divergence in plasticity in parental care between

selection lines. Alternatively, it could be that the sample size of the current study was not sufficient to detect genetic variability in plasticity.

These results contrast with the alternative hypothesis that females would use a bet-hedging strategy, producing offspring of a range of sizes regardless of the social environment they experience, with no change in mean larval mass (Simons 2009; Simons 2011). The social environment experienced by the female did not significantly affect within-brood variability in larval mass. This suggests that, if variation in larval mass is a form of bet-hedging, the extent of it does not alter depending on the social environment experienced. However, these results cannot rule out the possibility that the observed within brood variation in larval mass is adaptive bet-hedging, especially as the environment experienced in the wild can be highly variable. Adaptive phenotypic plasticity and bet-hedging are not mutually exclusive, and it is possible that burying beetle parental behaviour involves a combination of both strategies. However, a recent study on germination of the plant *Lobelia inflata* found a negative correlation between plasticity (differences in germination rate across temperatures) and potential bet-hedging traits (germination fraction and within-parent variation in timing of germination), suggesting that there may be a trade-off between the two strategies (Simons 2014).

The relative size of the focal female to the rival female did not have a significant effect on any of the measures of parental care or larval performance, with the exception of average larval mass for which the interaction between focal female line and the relative size of the focal female was only marginally non-significant. These results are consistent with those of a study on male burying beetles which investigated whether the experience of interacting with other males prior to competition over a carcass altered contest behaviour. It was predicted that males with this social experience would be able to gauge their own relative size in advance of the contest, meaning they have less to gain from being highly aggressive in the contest itself. In contrast, it was predicted that naive individuals would be more aggressive during contests so as to gain information on their relative size. However, it was found that social experience influenced male encounter rate but not aggression during contests over carcasses, suggesting that males do not gain information on their own size through social

experience (Lee et al. 2014). Therefore, in this present study, the females may have been able to detect the presence of a rival, and hence alter their parental behaviour, but unable to retain and use information on their own relative size. This is also consistent with the Pilakouta et al. (2016) study, which found that contest outcome, which is almost always won by the larger individual, did not affect female burying beetle parental care or larval performance.

In this study, low mating rate line females were significantly more likely than those from the high mating rate line to successfully produce at least one larvae surviving to dispersal. This was unexpected, but could be due to high mating rate line females experiencing more stress (e.g. due to more time spent interacting with the male), making them less likely to lay eggs, more likely to cannibalize larvae (Bartlett 1987), or giving them less time to prepare the carcass prior to larvae hatching. The coefficient of variation of larval mass was significantly lower for low mating rate line females than for high mating rate line females. This could possibly be due to a high mating rate being costly (Head et al. 2014), reducing the quality of the female's parental care and ability to distribute food evenly to larvae and reduce sibling competition. Larvae of low mating rate line females took significantly less time to disperse from the carcass than those from high mating rate line females, providing further support for there being a cost to high mating rate. The selection line of the female did not have a significant effect on duration of parental care, number of larvae surviving to dispersal or mean larval mass. This contrasts with the results of the experiment of Chapter 2 of this thesis, in which the offspring of low mating rate line individuals were more likely to survive to dispersal. This discrepancy could be due to differences in the methodologies of the two experiments. For instance, in Chapter 2 the focal adult was given a fixed number of 20 larvae, whereas in this present experiment the number of starting larvae was not controlled. The use of a fixed number of starting larvae in Chapter 2 may have resulted in a greater difference between high and low mating rate line individuals in the number of larvae surviving to dispersal. High mating rate line individuals may have been given more larvae than they could manage, reducing overall larval performance as their parental care is spread too thinly. In the experiment in this current chapter, focal females spent 48 hours with the carcass prior to breeding, in which they could start to prepare and bury it. This extra time to invest in pre-

hatching care could have reduced the difference between high and low mating rate line females in the number of larvae surviving to dispersal. It is also possible that the sample size in this present experiment was insufficient to detect a difference between selection lines in the number of larvae surviving to dispersal. The results of this current experiment also contrast with those of the Head et al. (2014) study on the same selection lines, which found that females from the low mating rate line provide care to larvae for longer and had higher offspring performance than those from the high mating rate line. However, unlike this study, Head et al. used a composite measure of larval performance, consisting of mean larval weight, development time and proportion of larvae surviving to dispersal, which may explain the difference between these results. In addition, Head et al. also included between-line crosses, meaning that females from each line were exposed to males from a range of genetic background instead of just one. This may have made the difference in the behaviour and performance of females from the different mating rate lines more apparent than in the current study.

Future studies could investigate the correlation in the wild between the competition environment experienced by adult burying beetles and that experienced by their adult offspring. This would reveal whether the increase in larval mass for offspring of females that experience competition is an adaptive anticipatory parental effect or a by-product of a reduction in brood size due to the costs to the female of competition. It would also be informative to investigate in detail the effect of competition over access to carcasses prior to reproduction on egg production. Further research could also focus in detail on whether increased competition results in female burying beetles displaying terminal investment (individuals with a low residual reproductive value showing an increase in investment in the current reproductive round rather than conserving resources for future reproduction). Pilakouta et al. (2016) found that females that experienced competition increased investment in the current brood, but did not investigate its impact on future breeding rounds. There is evidence for terminal investment with increased age in a related burying beetle species, *Nicrophorus orbicollis*. Older first time breeding females produce more larvae surviving to dispersal and use less of the carcasses for maintaining their own body mass than younger females (Creighton et al. 2009).

In conclusion, this study provides evidence for plasticity in female burying beetle parental care in response to the social environment. Females that interacted with a rival female prior to breeding produced significantly heavier larvae than isolated females. Whether this is an example of adaptive anticipatory parental effects could be examined in future studies by investigating the correlation between the social environment experienced by a female and her adult offspring. These results contrast with those of Pilakouta et al. (2016), which found that females that experienced a rival prior to breeding produce larger broods but larvae of the same average mass to those that haven't experienced a rival. In this current study, there was no evidence for genetic variability in this plasticity (a GxE), suggesting this plasticity would not respond strongly to selection. Knowledge of plasticity in parental care in response to the social environment is important for our understanding of its evolution and expression (Royle et al. 2014). Further studies could therefore investigate why there is a lack of genetic variability in the extent of plasticity of some traits. This study furthers our understanding of the consequences of female-female competition in burying beetles. It also confirms a previously reported trade-off between larval number and mass in burying beetles (Monteith et al. 2012). More generally, this study emphasises that parental behaviour can be plastic in relation to variation in the social environment, and how this can impact fitness. This study demonstrates the importance of investigating the long term fitness consequences of female-female competition, the effects of which can become apparent after the departure of the rival individual. While these results cannot rule out observed variation in burying beetle larval mass as an example of bet-hedging, they do show that it does not vary with changes in the social environment. Bet-hedging traits are found in species across a wide range of taxa, meaning that it is important to have a greater understanding of the links between bet-hedging and plasticity

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CHAPTER 4: GENERAL DISCUSSION

THE IMPORTANCE OF THE SOCIAL ENVIRONMENT FOR BURYING BEETLE PARENTAL CARE BEHAVIOUR AND OFFSPRING FITNESS

The social environment has important consequences for each stage of the burying beetle, *Nicrophorus vespilloides*, life cycle (Eggert & Müller 1997; Scott 1998). This study investigated the impact of two elements of the social environment on burying beetle parental care and offspring performance; mating rate and the presence of a same sex rival. In this chapter I first discuss the main findings of both experiments in this thesis. I then discuss the wider implications of these results for the study of mating traits, plasticity and parental care.

CHAPTER 2: IMPACT OF MATING RATE ON PARENTAL CARE AND LARVAL PERFORMANCE UNDER UNIPARERENTAL CONDITIONS

The experiment detailed in Chapter 2 investigated the impact of mating rate on parental care and offspring performance under uniparental conditions. This followed on from a previous study, Head et al. (2014), which had found that, under biparental conditions, females from a line selected for high mating rate provided less parental care and had lower larval performance than those from a line selected for low mating rate. However, selection on mating rate had no effect on male parental care and offspring performance, suggesting that, under biparental care, costs of a high mating rate are greater for females than males. Male parental care is known to be highly plastic, with more care provided in the absence of a female partner, meaning that the costs of a high mating rate for males may be more apparent under uniparental conditions (Smiseth et al. 2005). As predicted, the results of the experiment in Chapter 2 found evidence for a cost of a high mating rate for males and females, with individuals from the high mating rate line showing a reduction in number of larvae initially produced and proportion of larvae surviving to dispersal. Unexpectedly, mean larval mass was greater for high mating rate line individuals, which was probably due to a decrease in competition between larvae in smaller broods. Also following predictions, costs of a high mating rate were greater for females than males, as found by Head et al. (2014). Costs were also greater when breeding on a larger carcass, suggesting individuals experiencing a high mating rate are unable to take full advantage of the extra resources available. These results therefore

support the results of the study by Head et al. (2014) on burying beetles, and studies on other species (such as the spider *Stegodyphus lineatus* (Maklakov et al. 2005)), in finding a cost to a high mating rate on fitness. They also demonstrate the importance of investigating how the impact of mating on parental care and offspring performance depends on resource availability (such as carcass size), which has previously received little attention.

CHAPTER 3: PLASTICITY IN FEMALE PARENTAL CARE IN RESPONSE TO THE PRESENCE OR ABSENCE OF A RIVAL FEMALE

Understanding the evolution of plasticity of behaviour is vital, as it is believed to have important consequences for population survival in new habitats and speciation (Miner et al. 2005; Pfennig et al. 2010). Few studies have investigated whether there is genetic variability for the extent of plasticity (a GxE) of parental care traits in response to variation in the social environment (Royle et al. 2014). This is important, as if there is no GxE for plasticity in parental care traits the extent of plasticity will not respond strongly to selection, such as due to climate change (Nussey et al. 2007; Dingemanse & Wolf 2013). The impact of female-female competition on parental care and offspring performance has previously received little attention, and has largely focused on mammals. This was partly due to the fact that in many species females have less pronounced weapons (e.g. antlers) and ornaments than males, and tend to display less aggression (Clutton-Brock & Huchard 2013). This meant that female-female competition was seen as being less intense than male-male competition. However, female-female competition has increasingly received more attention, as studies have demonstrated that it can have a large effect on female fitness (e.g. in meerkats, *Suricata suricatta* (Clutton-Brock et al. 2006). A recent study on burying beetles found that females that experience the presence of a rival female prior to breeding produce larger broods, suggesting that competition can impact female parental care in this species (Pilakouta et al. 2016). The second experiment in this thesis, detailed in Chapter 3, investigated whether female burying beetle parental care is plastic in response to the presence or absence of a rival female.

As Pilakouta et al. 2016 had found that the presence of a rival female can increase a focal female's parental care, I predicted that females in my

experiment would be able to adaptively match the phenotype of their offspring to the competition environment they are likely to experience as an adult. Parental care can have a large positive effect on larval mass, which in turn has a significant impact on adult size and competitive ability (Otronen 1988; Eggert & Müller 1997; Scott 1998). I therefore predicted that females that experienced competition would produce heavier offspring. This was supported by the results of my experiment, as females that experienced a rival prior to reproduction produced heavier larvae. This could be an example of an anticipatory parental effect, although a recent meta-analysis suggests that these are rare (Uller et al. 2013). Further studies could investigate the correlation in the wild between the competition environment experienced between a female and her adult offspring, to confirm that this plasticity in parental care is adaptive. This increase in larval mass for females that experienced competition was accompanied by a decrease in brood size, further supporting the existence of a trade-off between offspring mass and number (Smiseth et al. 2014). There was no evidence for genetic variability, a GxE, in this plasticity in parental care.

A second strand to this experiment was to investigate the impact of the competition environment experienced on within-brood variation in larval mass, a potential bet-hedging trait. While this experiment could not rule out this trait as being an example of adaptive bet-hedging, it did reveal that the extent of within-brood variation in mass was not dependent on the social environment experienced by the female.

Altogether, this experiment demonstrated that the competition environment that females experience can impact parental care and larval performance, supporting the results of Pilakouta et al. (2016). It also suggests, due to the lack of a GxE, that the extent of plasticity in response to variation in the social environment of parental care traits may not respond strongly to selection.

PLASTICITY IN PARENTAL CARE AND ANTICIPATORY PARENTAL EFFECTS

An unexpected result from the experiment in Chapter 3 of this thesis was the apparent absence of a GxE (a gene-by-environment interaction) for plasticity in female parental care behaviour. This was slightly surprising given that the high

and low mating rate selection lines used in the study had been isolated for 40 generations, although it could be due to the sample size being too small to detect a GxE. For the extent of plasticity of a trait to evolve in response to selection there needs to be genetic variability for it (a GxE) (Nussey et al. 2007; Dingemanse & Wolf 2013). Therefore, the absence of a GxE in this study suggests that the extent of plasticity wouldn't respond strongly to future selection. This could potentially be the result of strong selection on parental care traits reducing genetic variation for plasticity, which would indicate the importance of the social environment for the evolution of parental care.

Research on other species has also found an absence of a GxE for plasticity in parental care/offspring performance in relation to the social environment. For example, a study on least killifish, *Heterandria formosa*, also found an absence of divergence between two populations in the extent of plasticity in the size of offspring produced at different population densities (Leips et al. 2009). It could be useful therefore to further investigate what causes the extent of plasticity to be conserved between different populations of a species. Leips et al.(2009) suggest that in their study this may be due to either a) the same level of plasticity being adaptive in both populations b) a reduction in plasticity of a trait being slow, or c) due to changes in the plasticity of a trait being constrained by genetic correlations with other traits. All of these could also potentially apply in this current study for the lack of a GxE for plasticity in parental care.

This lack of genetic diversity for plasticity in parental care has wide implications. For example, a change in the environment (such as due to climate change), could result in a change in the optimum level of plasticity in parental care in response to variation in the social environment. If a species cannot evolve in its behaviour quickly enough in response to a change in the environment, this could potentially lead to extinction, especially given the importance of parental care for parent and offspring fitness. It also has implications for the coevolution of behaviour expressed in pairs of individuals, such as parents and offspring. For example, if there is no GxE for plasticity in parental care it won't coevolve with plasticity in offspring begging behaviour. Another example of the consequences of a lack of a GxE for plasticity in parental care could include an impact on speciation (Pfennig et al. 2010). Plasticity in behaviour can allow

individuals to colonise a novel environment. This plasticity can then be selected upon, leading to the population becoming adapted to the novel environment through genetic accommodation (Crispo 2007; Pfennig et al. 2010). For example, conditions in the novel environment may be harsh, leading to parents always expressing high levels of parental care, regardless of the social environment. Over time, this may result in reproductive isolation between populations in the old and novel environment, and hence promote speciation. If there is no GxE for plasticity of a behaviour it won't evolve quickly through genetic accommodation, and hence plasticity may not promote speciation.

Chapter 3 also investigated whether female burying beetles, through their plasticity in parental care, can adaptively match their offspring's phenotype to the competition environment they are likely to experience. If so, this would be an example of an anticipatory parental effect, and would result in offspring having highest fitness in the environment predicted by the environment experienced by their parent. Anticipatory parental effects occurs when parents pass on information to offspring about the environment they are likely to experience, changing offspring phenotype to increase their fitness in that environment (Marshall & Uller 2007; Uller et al. 2013). I predicted that females that experienced the presence of a rival prior to reproduction would produce larger offspring, which would likely be better able as adults to compete for access to a carcass on which to breed. This prediction was supported, with females in the rival treatment group producing heavier but fewer larvae. This result contrasts with that of a recent meta-analysis of 58 studies searching for anticipatory parental effects, which suggested that they are rare (Uller et al. 2013). The next step would be investigate the correlation between the competition environment experienced by a female and her offspring, and the relationship between body size and fitness in different competition environments, to confirm this is an example anticipatory parental effect. This is important as, according to the meta-analysis discussed above, few studies investigate whether there is a correlation between the conditions experienced by parents and offspring for the environment they are manipulating (Uller et al. 2013; Burgess & Marshall 2014). Anticipatory parental effects involve plasticity in the parent's behaviour (in response to the external environment) and the offspring's behaviour (in response to the parent's phenotype). It is as yet

unknown whether anticipatory parental effects evolve primarily through changes in plasticity of the parent or offspring behaviour (Uller 2012). However, the absence of a GxE for plasticity in parental care behaviour in Chapter 3 suggests that anticipatory parental effects may evolve largely through changes in offspring plasticity.

MATIING TRAITS AND PARENTAL CARE

One of the findings of the experiment in Chapter 2 was that the effect of the costs of a high mating rate on parental care and larval performance were greater when breeding on a larger carcass. This suggests that high mating rate line individuals were unable to take full advantage of breeding on a larger carcass. This indicates that the coevolution of mating and parental care traits in males and females will be dependent upon the non-social environment experienced. For example, increased population density or a male biased sex ratio (such as due to the prevalence of a disease that has a higher mortality rate for females than males), could lead to increased competition between males for access to females, and result in selection on males for an increased mating rate to reduce sperm competition. In some habitats large carcasses may be more prevalent, and the selection pressure on females to express lower levels of parental care (due to this increased mating rate) could be greater than in a habitat with a tendency for smaller carcasses. This highlights the importance of measuring the effect of the social environment on parental care across a range of different non-social environments. Studies on other species have also found similar results; for instance, costs of a high mating rate reduce egg production in *Drosophila melanogaster* only when there is high food availability (Chapman & Partridge 1996). Future research could investigate in more detail interactions between the social and non-social environment on parental care and offspring performance. For instance, how common is it for the impact of the social environment on the expression of behaviour to be affected by the non-social environment? This would require information on the impact of the non-social environment on the effect of numerous different social environments on parental care . For example, does carcass size affect the impact of the parental care of a partner on an individual's parental care? Due to the ease at which the social and non-social environments can be manipulated in burying beetles, they are an ideal species in which to examine this topic.

WITHIN-BROOD VARIATION IN LARVAL MASS AND BET-HEDGING

The two experiments in this study differed in their results with regard to whether selection on mating rate impacts within-brood variation in larval mass; selection line (high or low mating rate) had a significant effect on the coefficient of variation of larval mass in the experiment in Chapter 3 but not in Chapter 2. In Chapter 3, high mating rate line females showed greater variation in larval mass than those from the low mating rate line. This suggests the costs of a high mating rate may prevent the female from distributing food and/or parental care in a way to reduce this variation. Selection line may not have had a significant effect on the coefficient of variation in the experiment in Chapter 2 for various reasons. The experiment in Chapter 2 involved broods raised by males as well as those raised by females, while Chapter 3 focused only on female parental care; if selection line had no effect on the coefficient of variation of larval mass for broods raised by males, an effect of selection line could be more difficult to detect. In Chapter 2, carcass size and the proportion of time spent caring by the parent had a significant effect on the coefficient of variation of larval mass, suggesting that lower sibling competition for resources (i.e. when on a large carcass) and proportion of time providing parental care reduced within brood variation in mass. Neither the effect of carcass size or parental care on within-brood variation in larval mass were tested in the experiment in Chapter 3, so these results could not be confirmed. Overall the experiments of this thesis suggest that a) parental care can decrease within-brood variation in larval mass b) selection for a high mating rate may increase within brood variation in larval mass due to the costs a high mating rate impacting parental care.

The causes and fitness consequences of within brood variation in offspring mass could be an informative area of further study. Studies on other species have found that elements of the non-social environment can increase within-brood variability in offspring mass. In one study, sand martin (Riparia riparia) broods that were exposed to ectoparasites showed greater within-brood variation in offspring mass than those in which ectoparasites were absent, but there was no difference overall between treatments in mean offspring mass (Szep & Moller 2000). The authors of the study suggest that increasing within-brood variation in offspring mass in response to ectoparasites may be an adaptive response by the parent; this could be due to the parasite load being

concentrated on a small number of the nestlings, increasing the performance of the remainder of the brood.

It would be useful to investigate whether within-brood variation in larval mass in burying beetles is an example of a diversification bet-hedging trait. These are traits which, although they may not have the highest fitness over any one generation, have high long term fitness by having low variation in success between generations (Simons 2011). Evidence for within-brood variation in burying beetle larval mass being a bet-hedging trait comes from the fact that, while larger individuals nearly always win access to the carcass, a recent study indicated that smaller individuals may be better able to locate carcasses and not attract competition (Hopwood et al. 2016). It is unknown whether the extent of bet-hedging traits can vary with the environment experienced. For instance, in some environmental conditions the future environment may be very predictable, and the need for bet-hedging may be low. In contrast, in other environments the reverse may be true, and the extent of bet-hedging may be increased. The aim of Chapter 3 was therefore to investigate whether the extent of within-brood variation in larval mass varies with the social environment (presence or absence of a competitor) experienced by the female. I predicted that the presence of a rival female prior to breeding would indicate higher uncertainty about the competition environment that a female's offspring would experience than the absence of a rival. I therefore expected that within-brood variation in larval mass would be greater for females in the rival treatment group. This was not the case, as the social environment experienced by the female had no effect on within-brood variation in larval mass. This implies that, when diversification bet-hedging is used as a strategy to respond to a variable environment, the extent of diversification is not affected by the social environment experienced.

LONG LASTING CONSEQUENCES OF FEMALE-FEMALE COMPETITION

To date, most studies on the effect of competition with same sex rivals on parental care and mating traits have been on vertebrate species (especially mammals), and have focused on males (Stockley & Campbell 2013; Clutton-Brock & Huchard 2013). The results of the experiment in Chapter 3 of this thesis demonstrate that female-female competition can have long lasting implications for behaviour and fitness in a non-vertebrate species. In this

experiment the rival burying beetle female was only present prior to the start of parental care, yet this still resulted in the focal female producing heavier, but fewer, larvae on average. Pilakouta et al. (2016), carried out a similar experiment on burying beetles and also found that the presence of a rival can influence later parental care and offspring performance. They hypothesise that the presence of a rival individual prior to breeding may act as a signal of high population density. However, their results differ from those of the current study in finding that, rather than increasing offspring mass, females with experience of interacting with a rival shift towards producing more offspring, potentially investing more in the current brood as competition to breed in the future may be fierce. An earlier study on a related burying beetle species, *Nicrophorus orbicollis*, found that at higher population densities there is a shift towards producing heavier (but fewer) larvae which will be better able as adults to compete for a carcass, supporting the results of Chapter 3 (Creighton 2005). Therefore, there would appear to be evidence for two different possible responses of female burying beetles to high population density/high competition environment; invest more in the current brood by producing more larvae, as future breeding opportunities for the adult may be limited (Pilakouta et al. 2016), or produce heavier larvae in the current brood as they will face greater competition as an adult (Chapter 3).

Further untangling these questions regarding investment in offspring size vs number and investment in the current brood vs future broods will be informative, not just about burying beetles but much more broadly, as many species across a broad range of taxa can regulate offspring size and number. For example, a study on least killifish, *Heterandria formosa*, found that females produce larger offspring at higher population densities (Leips et al. 2009). A recent study on banded mongooses, a cooperatively breeding species, found that females increase prenatal investment in offspring (by increasing foetus size) when there is greater competition from other breeding females (Inzani et al. 2016). This effect on prenatal investment was greater when rainfall levels were poor, which is likely due to competition being increased in this environment. While increasing prenatal investment when there is increased competition would appear to be adaptive, no link between foetus size and pup size or survival was found. The authors of the study therefore suggest that the impact of prenatal

investment may become apparent at a later stage of the offspring's life. This therefore highlights the importance of investigating the long term consequences of competition.

It is possible that the extent of female-female competition is constrained due to a trade-off with parental care (Clutton-Brock 2007; Stockley & Campbell 2013). In Chapter 3, females that interacted with a rival tended towards producing a smaller number of heavier larvae than those that didn't. It is possible that interacting with a rival reduces the number of eggs that a female can produce or the number of larvae she can care for, and this leads to an increase in larval mass due to decreased competition between larvae. However, there was no effect of the presence or absence of a rival female on the proportion of time the female cared for larvae. Pilakouta et al. (2016) found that females that interacted with a rival produced as many eggs as those that didn't, spent more time providing direct care, and produced larger broods. In addition, they also found that whether a female won or lost the contest for the carcass had no effect on her parental care or offspring performance. Altogether this suggests that competing with a rival for access to a carcass is not very costly for female burying beetles, and investment in competition may not be strongly constrained by a need to invest in parental care. This may be because contests don't escalate, as the outcome in this species is usually determined by body size, and the loosing females can have some reproductive success by acting as a 'brood parasite' female, laying her eggs surrounding the carcass (Eggert & Müller 1997).

CONCLUSION

The experiments detailed in this thesis examined the effect of two elements of the social environment on parental care and offspring performance in burying beetles. The experiment in Chapter 2 investigated the impact of mating rate on parental care under uniparental conditions. As predicted from Head et al. (2014) who carried out a similar experiment with biparental conditions, the results found that the costs of mating on offspring performance are greater for females than males. Few studies have investigated the impact of female-female competition on parental care and offspring performance. In Chapter 3, female burying beetle parental care and offspring performance was found to be plastic

in relation to the presence or absence of a rival female. Females that experienced a rival prior to reproduction produced heavier larvae, which are expected as adults to be better able to compete for access to a carcass on which to breed. This may therefore be an example of an anticipatory parental effect, which are believed to be rare (Uller et al. 2013).

An important finding of Chapter 2 was that the costs of a high mating rate depend upon the non-social environment (carcass size) experienced. This suggests that the evolution of parental care in response to changes in mating traits, and vice versa, will depend on the non-social environment. In Chapter 3, there was no GxE for plasticity in parental care in relation to variation in the social environment. This implies that the extent of plasticity of parental care traits in response to variation in the social environment may be slow to respond to selection. The implications of this include populations potentially being unable to respond to climate change by changing their parental care behaviour. Within-brood variation in burying beetle larval mass could be an example of a diversification bet-hedging trait. The results of Chapter 3 indicated that the extent of within-brood variation in larval mass does not depend on the social environment experienced by the female. This suggests that bet-hedging traits remain consistent across environments. There is still much to learn regarding the impact of the social environment on the expression and evolution of parental care.

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